Minor Neurological Dysfunction in Healthy Children Born at Term

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evidence for a sex-specific significance

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Chapter 1

Introduction

With the increase of possibilities for imaging studies, attention for developmental disorders and the underlying neural substrate increased. This has also led to increased attention for minor neurological dysfunction, which may be defined as the occurrence of neurological dysfunction in absence of evident pathology. Minor neurological dysfunction (MND) may manifest itself in subtle coordination problems, fine manipulative disability or dysfunctional posture and muscle tone. However, the implications of having MND are not yet clear. The aim of this thesis will be to contribute to knowledge on the clinical significance of MND, which is of importance as an understanding of co-occurring problems may also provide opportunities for intervention.

In this chapter, first a short overview will be provided on typical functional neurological development and differences in the neurological development for boys and girls. Next, information regarding atypical neurological development will be provided: what is known about prevalences of various developmental disorders, such as attention deficit hyperactivity disorder and learning disabilities, their underlying neural substrate and the early risk factors for these disorders. Sex differences in developmental disorders will also be mentioned. Finally, evidence up to date for the significance of MND will be reviewed and an overview of the thesis will be given.

Neurological development

In order to understand developmental disorders, we must first have understanding of typical developmental processes in the brain.

Functional brain development

Development of motor performance and speed (1) and cognitive functions, such as working memory (2) is a gradual and long lasting process; at nine years of age, a child has more skills in his or her repertoire than for example a four year old child. The development of increasingly complex skills is the result of experience and ongoing maturation of the brain. Animal research has demonstrated that neuronal proliferation, growth of axons, dendrites and synapses, myelination of axons as well as regressive phenomena such as neuronal cell apoptosis, axon retraction and synapse elimination continue after birth with some of the processes lasting throughout adolescence and adulthood (3-6).

Due to the development of imaging techniques such as MRI, information regarding brain development in humans has become more readily available. Imaging studies in humans have shown that the developmental trajectories of different brain regions follow a different course. This is demonstrated by different ages at which the

brain regions reach peak sizes. Peak sizes of brain areas are the result of an "inverted U" developmental trajectory of the grey matter of these brain regions (7,8), meaning that the amount of grey matter decreases after having reached peak volumes as a result of the above mentioned regressive phenomena. Ages at which these peak sizes occur are for example approximately 8.5 years of age for the frontal cortex (7), between 7.5 and 10 years for the basal ganglia (8), between 9 and 11 years of age for the hippocampus and amygdala (9); for the prefrontal cortex peak sizes are reached at approximately 11.5 years of age (7), for cerebellar volume at approximately 12 years of age in girls and 15.5 years in boys (10) and finally the temporal cortex, which reaches peak sizes at approximately 17 years of age (8). In general, brain regions involved in sensorimotor functions (for example the precentral gyrus in the frontal lobe) mature before brain regions involved in higher-order functions, such as the temporal cortex and prefrontal cortex (7).

For the execution of brain functions, among which cognitive and motor functions, activation of distributed brain systems is required. For example, attentional switching makes use of a number of brain regions, namely the anterior cingulate, the supplementary motor area, the orbitofrontal and dorsolateral prefrontal cortex and of the basal ganglia and thalamus (11). Another example comes from activation of fronto-striatal and fronto-cerebellar circuits, which are seen in tasks of executive function and working memory (12-14). These brain circuits are also associated with complex motor tasks: the cortico-striatal system with movement control and sequential finger movement tasks (15,16) and the cortico-cerebellar system with motor learning, adaptation and timing of movements (14,16). Due to the protracted development of the involved brain regions and their connectivity, it can be expected that the cognitive functions subserved by these brain networks, also continue to mature until adulthood.

Sex differences in typical neurological development

There is increasing evidence for sex differences in both brain structure and function. It has been demonstrated for example, that girls perform better on verbal tasks, whereas boys perform better on tasks of visuospatial ability (17). These sex differences in brain function may be the results of sex differences in structural neurological development, for which there is increasing evidence.

Sex differences in developmental trajectories

As mentioned above, the developmental trajectories of grey matter volume may described by an inverted U shaped curve. This is true for both boys and girls, however, peak sizes are reached at different ages. Total cerebral volume peaks at approximately 10.5 years of age in girls, whereas in boys peak sizes are reached at 14.5 years (18); the peak volume of the grey matter is reached at 8.5 and 10.5 years respectively (18). Differences in developmental trajectories can also be found for separate brain regions, with girls reaching peak sizes before boys. For example, caudate nucleus volume peaks at 10.5 as opposed to 14 years, the cerebellum reaches peak sizes at approximately 12 years and 15.5 years, grey matter of the frontal lobe at 9,5 and 10.5 years, the parietal lobe at 7.5 versus 9 years and the temporal lobe at 10 years and 11 years of age in girls and boys respectively (18). This implies that girls reach neurological maturity before boys with respect to grey matter development. Sex differences may also be found in development of the amygdala and hippocampus. A study demonstrated that hippocampal volume increases more in females, whereas volume of the amygdala increases more in males in children aged 4 to 18 years (19,20).

Regarding white matter development, the amount of white matter increases during until adolescence in both boys and girls; however, this increase is larger in the male brain compared to the female brain. This applies not only to total amount of white matter, but also to the white matter volume in the frontal lobes, temporal lobes, parietal lobes, occipital lobes and corpus callosum (18,21).

Sex differences in brain volume

Apart from differences in the developmental trajectories of the brain, differences have also been demonstrated with respect to brain volume. Both in vivo as well as post mortem studies in children and adults have consistently demonstrated that the male brain is approximately 10-12% larger than the female brain (18,21-24). After adjusting for this difference in overall brain volume, differences in regional brain volumes have also been demonstrated, although conflicting results have been published. However, in general it seems that females have increased cortical thickness of the right lateral parietal and temporal regions (25) and larger volume of the hippocampus and the caudate nucleus of the basal ganglia compared to males (26,27). In contrast, males have relatively larger amygdala (26), and cerebellum (22,28).

Developmental disorders

Developmental disorders refer to disorders which are usually first diagnosed during childhood or adolescence and encompass disorders of psychological or motor development, which may include problems with behaviour, language, attention, socialization and motor problems, such as problems with coordination or fine manipulative ability.

Prevalence of developmental disorders

Prevalences of developmental disorders during childhood are provided in table 1. Of particular interest are sex differences in these prevalences of the developmental disorder. It appears that boys are more vulnerable for most developmental disorders. Increased odds can be found for boys for pervasive developmental disorders, such as autism and PDD-NOS (29-31), disruptive behaviour disorders, such as attention deficit/hyperactivity disorder (ADHD), oppositional defiant disorder and conduct disorder (29,32-34), and developmental coordination disorder (35). Girls on the other hand, seem to be more vulnerable for mood disorders, such as anxiety and depression, and for eating disorders (32-34).

Furthermore, the prevalence of developmental disorders varies across different age groups. At 9 to 10 years of age, the prevalence of psychiatric disorders is especially high, compared to other age-groups (33). In particular, the prevalence of childhood disorders, such as autism, ADHD and tic disorders is higher before adolescence than thereafter; after puberty the prevalence of these disorders decreases (36); For instance, the prevalence of ADHD is 2.2% in 9-year old children, compared to 0.3% in 16-year old children (33). On the other hand, the prevalence of other disorders, such as depression, anxiety disorders, eating disorders and substance abuse, tends to increase after puberty (32,33,36). For example, the prevalence of any depressive disorder is 0.5% in 9-year old children, compared to 3.2% in 17-year old children (33).

Of the children with a psychiatric diagnosis, 25-30% has more than one diagnosis (32,33). Comorbidity occurs in particular for anxiety disorders, depressive disorders and disruptive behaviour. More than half of the children with ADHD also have other behavioural disorders, and in particular other disruptive disorders, such as oppositional defiant disorder or conduct disorder (32,37), but also mood disorders, anxiety disorders and learning disabilities are frequently encountered in children with ADHD (37). Furthermore, developmental motor disorders also co-occur with behavioural disorders and learning difficulties. For example, children with DCD often also have reading disability or ADHD (38), but also an increased risk for depression and emotional problems (35).

Table 1. *Prevalence (%) of developmental disorders during childhood*

		Total	Boys	Girls
Pervasive developmental disorders				
	Autism (31,39,40)	0.2–0.3	0.3–0.5	0.1–0.2
	Asperger (31,39)	0.1	0.2	<0.1
	PDD-NOS (31,39)	0.1–0.4	0.4	0.1
Behavioural disorders				
	ADHD (32-34,39)	0.9–2.1	1.5–2.3	0.3–0.5
	Oppositional defiant disorder (32-34,39)	2.5–2.8	3.1–3.2	2.1–1.4
	Conduct disorder (32-34,39)	0.5–3.3	2.1–4.2	0.8–1.2
Mood disorders				
	Any anxiety disorder (32-34)	2.4–8.6	2.0–3.5	2.9–4.0
	Any depressive disorder (32,33)	0.3–3.4	0.9–1.6	1.0–2.8
Learning disabilities				
	Reading difficulties (41)	19.9	21.0	18.9
	Arithmetic difficulties (41)	10.3	7.3	12.9
Motor disorders				
	Cerebral palsy CP (42,43)	0.2–0.4	0.4	0.3
	Developmental coordination disorder (44)			
	Moderate	4.9	8.0	1.1
	Severe	8.6	12.9	3.2
Tic disorders				
	Verbal tics (33)	0.4	0.6	0.1
	Motor tics (33)	2.0	2.1	1.8
	Gilles de la Tourette (33,39)	0.1–0.2	0.1	0.2
Other				
	Eating disorders (32)	<0.1	<0.1	0.2
	Substance use disorder (33,34)	1.7–5.3	2.8	2.0

Neural substrate of developmental disorders

With the increase in neuroimaging studies, information regarding the neuroanatomical substrate of developmental disorders has also become more available, although the precise neural substrate remains unknown and studies have provided inconsistent results. Frequently, in children with developmental disorders, a focal neural lesion can not be found. It is more plausible that dysfunction of complex neural systems are the cause of developmental disorders. Here we will focus on the findings in ADHD and learning disabilities.

In children with ADHD, MRI studies have demonstrated smaller volumes of the cerebellum, globus pallidus, caudate nucleus, right prefrontal brain regions, (anterior regions of) the corpus callosum and smaller white matter volumes of the parietal/occipital regions compared to children without ADHD (45-47). Further support for involvement of frontal brain regions and the caudate nucleus comes from functional imaging studies, reporting different activation of the frontostriatal circuitry in children with ADHD compared to typically developing children, which has been hypothesized to be due to a delay in maturation of this circuitry (48). This is supported by the finding of frontostriatal hypoperfusion during a response inhibition task in adolescents with ADHD (49) and lower cerebral glucose metabolism in the striatum, thalamus, cingulate, hippocampus and frontal lobe in adults with ADHD (46). These findings are in concurrence with the idea that the prefrontal brain is involved in cognitive functions such as planning, working memory, attention and impulse control; functions which are impaired in children with ADHD. The results of imaging studies in ADHD are further in line with the hypothesis that frontal-striatal circuits are activated in the initiation and execution of complex cognitive and motor functions/ executive functions (14).

In children with language and reading disorders, studies have reported differences in brain volume compared to typical children: smaller volume of the cerebral cortex, thalamus and caudate nucleus and larger volume of the temporal and posterior-parietal regions have been reported (46,50). Specifically a lot of attention has been paid to the planum temporale, a landmark located on the superior temporal lobe: the majority of typically developing children demonstrate left > right asymmetry regarding size of the planum temporale. In children with developmental reading disorder, this asymmetry is absent (46,51). In concurrence with these findings of abnormal lateralization, is the finding of an absence of an increase in left hemisphere cerebal blood flow during a verbal task in children with expressive-receptive language disorder (52); more specifically, children with developmental dyslexia show less activation of the prefrontal and temporal cortex during phonological processing compared to

typically developing children (53). Finally, differences in volume of the corpus callosum and increased white mater volumes have been demonstrated in developmental language disorders (54,55), in particular of the frontal and temporal lobe (54).

In summary, it can be assumed that it is not dysfunction of a specific, focal brain region that underlies the neural substrate of major part of the discussed developmental disorders. It is more likely that these developmental disorders can be attributed to dysfunction in complex, interacting brain systems.

Early risk factors for developmental disorders

Children with developmental disorders thus seem to have a different neurological development compared to typically developing children. What may be the causes or risk factors for atypical development and thus for developmental disorders?

It is well recognized that both genetic and environmental factors play a role in the manifestation of developmental disorders, making the aetiology of developmental disorders multifactorial. In particular ADHD and learning disabilities have a strong genetic component; the evidence for genetic origin coming mostly from twin studies, which have demonstrated a heritability of ADHD of 76%, meaning that this proportion of variation in behaviour may be attributed to genetic variation (56). In reading disabilities there is also a strong genetic component with heritability between 55% and 72% (57). However, genes do not account for the total risk for developmental disorders, as the heritability is not 100%, implying that environmental influences also play a role in developmental disorders. For example, prematurity (58), low birth weight (59,60), familial adversity (61) or lower socioeconomic status (62), maternal psychological stress (63) and anxiety (64) during pregnancy and exposure to substances such as lead (62,65), or prenatal exposure to alcohol or tobacco (60,65-67) are associated with childhood (symptoms of) ADHD or learning disabilities. The influence of environmental factors is additive to the genetic risk, but also interactive with genetic information. For example, prenatal exposure to alcohol (68) or nicotine (69) interacts with genes in the risk for ADHD, and parental education may interact with genes in reading ability (70). These gene-environment interactions suggest that the expression and penetrance of genes may be influenced by pre- or postnatal environmental factors or - the other way around - that genes moderate the environmental risk .

Minor Neurological Dysfunction

The finding that motor problems and problems in other neural domains, such as cognition and behaviour, often co-occur in developmental disorders (14), has led to an increased attention for MND. MND has been characterized as the occurrence

of neurological dysfunction in absence of apparent neurological pathology, such as cerebral palsy. It can be expressed in type and severity of MND. The type of MND is described in eight domains of dysfunction: fine manipulative ability, coordination, choreiform dyskinesia, posture and muscle tone, reflexes, associated movements, sensory deficits and cranial nerve function (71). The number of dysfunctional domains determines – at least prior to puberty - the severity of MND, which is described in the classifications simple MND and complex MND (72). Simple MND is considered to be the mild form of MND and can also be regarded as a non-optimal form of development, and may be described as a minor neurological *difference,* whereas complex MND is seen as the clinically relevant form of MND, and has an aetiology resembling that of cerebral palsy (72).

Prevalence of MND

The prevalence of MND varies across different age groups, among children born preterm and full term, as well as for boys and girls. For term born children the prevalence of MND is lower. At the age of 18 months, MND is found in 3-7% of children (73); at pre-school age approximately 10% of children have simple MND and 3% of children are classified with complex MND (74). At school age, there is an increase of the prevalence of MND: of children aged nine years 15% are classified as having simple MND and 6% of children are classified as complex MND. However, after the onset of puberty, the prevalence of MND decreases to 7% for both simple and complex MND. Furthermore, there is a sex difference in the prevalence of MND: both simple and complex MND occur twice as often in boys than in girls (72).

The variation in prevalence is thought to reflect the age-dependent increase in complexity of brain functions. Complex motor functions as well as higher order cognitive functions develop throughout childhood until adolescence. This is in line with the maturation of brain circuitries thought to underlie these functions, such as cortico-striato-thalamo-cortical and cerebello-thalamocortical tracts. The first of these tracts, cortico-striato-thalamo-cortical tract, has been associated with motor functions such as movement control and sequential finger movement tasks (15) and the latter, the cerebello-thalamocortical tracts with motor learning and timing of movements (14).The increasing complexity may give rise to more subtle dysfunctions, such as MND.

However, as is mentioned above, the prevalence of MND decreases after the onset of puberty. This decline is possibly the result of the hormonal changes, such as a substantial rise in estradiol and testosterone, occurring during puberty. The gonadal hormones can influence organization of the nervous system (75). For example, increased levels of pubertal testosterone and estradiol have been associated with volumes of the amygdala, hippocampus and parahippocampal grey matter volume

(76) as well as with white matter integrity (77). It seems conceivable that the rise in sex steroids caused by puberty, may also play a part in the decline of MND after the onset of puberty. Soorani-Lunsing et al (1993) reported that in approximately half of the 12 year old children having MND, MND had disappeared at 14 years of age (78).

Early risk factors for MND

Besides a genetic origin of MND, prenatal, perinatal and neonatal adversities can also be the cause of MND. Before birth, adversities in the intra-uterine environment, such as maternal drug or alcohol use during pregnancy, are associated with the development of MND (79). Drugs with neuroteratogenic characteristics that are associated with the development of MND include anti-epileptic drugs (80) and coumarins (81).

Besides exposure to neurotoxic substances, adversities such as low fetal weight, preterm birth and intra-uterine growth retardation are associated with the development of MND (82-85). For example, Arnaud et al. (82) reported that the prevalence of MND is higher in preterm born children than in full term born children: at five years of age, the prevalence of simple and complex MND in children born before 33 weeks of gestation was 41% and 3% respectively, compared to 22.0% and 0.7% in children born between 39 and 40 weeks of gestation. Furthermore, children born small for gestational age, whether born preterm or at term have increased risk for developing MND (86,87). This is corroborated by the fact that the presence of complex MND at school age is associated with absent or reversed end-diastolic flow in the fetal aorta (88). Children born prematurely or with very low birthweight are also at increased risk for developing brain lesions, such as intraventricular haemorrhage, periventricular echodensities or periventricular leukomalacia (89). Differences in regional brain volumes have also been demonstrated in children born preterm (90,91). These brain abnormalities are also a risk factor for the development of MND independent of gestational age. (92,93).

On the other hand, breastfeeding after birth appears to have a beneficial effect on the development of MND. Children breast fed after birth, less often had MND at nine years of age than children fed formula (94).

However, not all children exposed to perinatal adversities develop MND. Early markers for MND at later ages include the quality of general movements (GMs), which are complex movements involving the entire body characteristic for early infancy (95-97).

In particular the last phase of GMs, the so called fidgety GMs, predict neurodevelopmental outcome at school age. The studies showed that children with definitely abnormal GMs had a high risk for developing cerebral palsy; children with mildly abnormal GMs were at risk for developing MND. Of the children with mildly abnormal GMs in infancy, approximately half developed MND at school age. (98).

The quality of GMs in infancy can also predict specific types of MND at school age: infants with abnormal GMs have increased risk to develop fine manipulative disability and coordination problems at four to nine years of age (96,99).

MND and associated impairments

Presence of MND has been associated with motor performance at school age (83,100,101). For example, children scoring below the 5th percentile on the Movement ABC more often have complex MND, than children scoring between the5th and 15th percentile. Specifically, poor motor performance is associated with the neurological domains fine manipulative disability and coordination problems (101).

Furthermore, MND at school age has been associated with both learning and behavioural problems. Previous studies have demonstrated that complex MND is more strongly related to these problems than simple MND. Learning problems that have been associated with the severity of MND include impairments in spelling, reading and arithmetic skills (72,102,103). Specific types of MND can be identified in the relation with learning problems: fine manipulative disability, coordination problems, and to a lesser extent choreiform dyskinesia and dysfunctional posture and muscle tone have been associated with school failure and problems with mathematics, reading and spelling. (103,104).These types of MND have also been related to behavioural problems, such as attention problems, fearfulness, clumsiness, social problems and internalizing and externalizing behaviour (103-105).

Aim of this thesis

This thesis focuses on MND in school age children and the correlates of MND in the form of learning and behavioural problems. The study differs in the following two aspects from previous studies on MND and associated learning and behavioural problems:

– Previous studies have lacked in general standardized and normed tests for the evaluation of these learning and behavioural impairments. In this thesis, the association with learning and behavioural problems will be examined using assessments that have been standardized and normed for different age-groups, taking developmental changes into account.
– Previous studies included children born preterm or small for gestational age. As a result, relatively little is known about the implications of MND in low risk children born at term. The studies addressed in this thesis, excluded children with perinatal risk, and included only healthy, term born children.

Therefore, the aim of this thesis is to study the significance of MND in a healthy, full term born population without risk for developmental disorders, using standardized tests. Questions addressed in the thesis are:

– Are parental psychological distress or anxiety associated with MND in the child? As mentioned above, prenatal stress factors, such as preterm birth, intra-uterine growth retardation and maternal drug and alcohol use during pregnancy, are associated with MND at later age. However, little is known about the relation between of parental psychological distress and MND.
– What is the clinical significance of the presence of MND; or more specifically: is the presence of MND in healthy term born children associated with IQ, cognition and behaviour? Are specific types of MND associated with cognition and behaviour?
– Do sex differences occur in the associations between MND and IQ, cognition and behaviour?

Methods

The data of two projects running at the Institute of Developmental Neurology of the University Medical Center Groningen (UMCG) were used to answer these questions. The first project is the Groningen ART cohort, which offered the opportunity to assess relations between parental anxiety and well-being on the one hand and MND of infants on the other. The second project, the Groningen LCPUFA project, enabled us to assess neurocognitive and neurobehavioural associations. The nature of the two projects will be summarized below. In addition, a brief overview of the applied assessments is provided.

The Groningen ART cohort

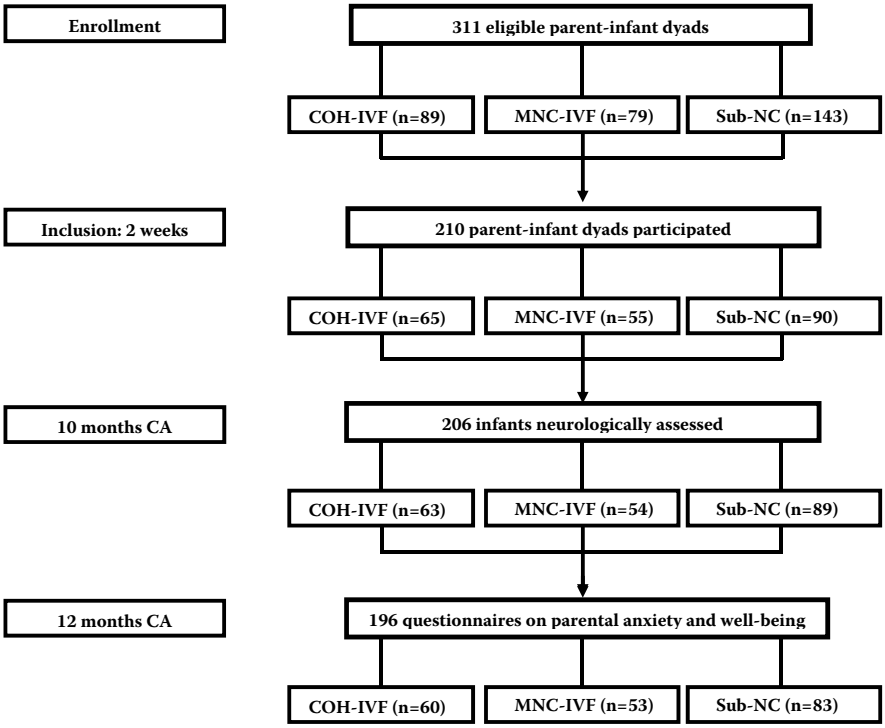
The Groningen ART cohort is a longitudinal study assessing relationships between assisted reproduction and neurodevelopmental outcome (74,106-108). Parent–child dyads were recruited at the department of reproductive medicine of the UMCG. Children were either conceived naturally in subfertile couples (Sub-NC; n=90), or by means of *in vitro* fertilization (IVF) (n=120). Children born after IVF, were born either after conventional IVF, that is with controlled ovarian hyperstimulation (COH-IVF) or born after IVF in the modified natural cycle (MNC-IVF). Twins and children born from cryopreserved or donor-gametes were excluded from the study. Two hundred and ten parent–child dyads were included at the infants corrected age of 2 weeks.

A neurological examination according to the Touwen Infant Neurological Examination (TINE) was performed in 206 infants at 10 months of age. With this examination, spontaneous motor behaviour is observed in supine, prone, sitting, standing and walking, and in addition muscle tone and reflexes are evaluated. The assessment gives an indication of function and dysfunction across different domains: reaching and grasping, gross motor function, visuomotor function, sensorimotor function and brain stem function. During the first year of life, TINE has a good inter-rater reliability (κ=0.83), sensitivity (91%) and positive and negative predictive power for neurological condition at 18 months, although the specificity for outcome at 18 months is moderate (67%) (109).

At the infants' age of 12 months, 196 parents answered questionnaires on anxiety and well-being, for which a Dutch version of the State-Trait Anxiety Inventory (STAI) (110), and the Dutch version of the General Health Questionnaire (GHQ) were used .The first provides a measure for trait anxiety: the characteristic of perceiving situations as threatening and reacting to these situations with increased anxiety, whereas the latter gives an estimation of the degree of psychological discomfort and the probability that mental health problems are present.

Participants and non-participants at 12 months were similar on background variables such as parental age and education and infants gestational age, birth weight, sex, and neurological optimality score at 10 months.

Flow chart of parent-infant dyads from the Groningen ART cohort: from study enrolment until 12 months follow-up.



Abbreviations: CA: corrected age; COH-IVF: controlled ovarian hyperstimulation IVF; MNC-IVF: modified natural cycle IVF; Sub-NC: subfertile – naturally conceived

The Groningen LCPUFA project:

The Groningen LCPUFA study was designed to examine effects of supplementation of formula with long chain polyunsaturated fatty acids (LCPUFA) during the first two months after birth. To this end, parents of participants were recruited between February 1997 until October 1999 from hospitals and midwife clinics in and near Groningen, the Netherlands. At birth, 474 infants born between 37 and 42 weeks of gestation were enrolled in the study. Exclusion criteria were children with a congenital disorder interfering with daily life, children from multiple gestations, adopted and foster children, children whose mothers did not master the Dutch language and formula-fed infants who had received breast milk for more than five days. The study population consisted of three groups: a group of

children whose mothers chose to breastfeed after birth (n=160), and two groups of formula fed children. The children who were formula fed after birth were randomized into two groups: one group receiving standard formula after birth (Nutrilon Premium; Nutricia, Zoetermeer, Netherlands; n=167) and one group who received supplemented with LCPUFA after birth (n=145). LCPUFA formula consisted of the standard formula supplemented with 0.45% (by weight) arachidonic acid (AA) and 0.30% (by wt) docosahexaenoic acid (DHA). After birth, extensive information on social background, obstetric conditions, and pre- and perinatal circumstances was collected

At nine years of age, 341 children participated (177 boys, 164 girls; 72% of the original cohort), and were examined using a neurological examination, an IQ-test and cognitive tests. Parents and teachers of children were asked to fill in questionnaires on behaviour.

Neurological examination

The examination of the child with MND allows for the detection of subtle neurological dysfunctions. This examination evaluates the child in terms of MND and is an age-specific assessment that takes developmental changes into account. The examination evaluates dysfunction in eight different domains: posture and muscle tone, reflexes, choreiform dyskinesia, coordination, fine manipulative ability, associated movements sensory deficits, and cranial nerve function. The criteria applied for the classification of dysfunctional domains have been defined by Hadders-Algra (71,111). The distinction between simple MND and complex MND is made based on the number of dysfunctional domains. Psychometric properties of the examination of the child with MND in general are good; interrater, intrarater, and test–retest reliability for neurological classification varied from good to excellent (71).

Cognitive and behavioural assessments

At the age of nine years a battery of cognitive tests was used, covering a broad range of cognitive functions. The battery consisted of 1) the Wechsler Abbreviated Scale of Intelligence (WASI) (112) to assess intelligence; 2) the developmental neuropsychological assessment (NEPSY) for the assessment of specific cognitive functions (113), 3) the test of everyday attention- children (TEA-Ch) to evaluate different types of attention (114) and 4) the children's memory scale for the assessment of memory function (115). Of the NEPSY, TEA-Ch and CMS a selection of tests were administered. The selection aimed to avoid overlap in the cognitive functions assessed, but also to limit the examination time for the children, to limit the effect of fatigue and loss of concentration on the test performance.

The WASI (112) is a validated screening tool used to evaluate various types of intelligence, including verbal knowledge, visual information processing, spatial and non-verbal reasoning, and general intelligence (112). Administration of the WASI results in three IQ scores: verbal IQ (subtests: vocabulary and similarities) and performance IQ (subtests: block design and matrices), which in combination form a full IQ score. Verbal IQ is a measure of crystallized intelligence, the knowledge a person has acquired during his or her life. Performance IQ on the other hand, gives an estimate of fluid intelligence: the ability to reason, to think in an abstract way and to solve problems. Although the WASI is an abbreviated test used for screening purposes, it correlates well with more comprehensive intelligence tests, such as the Wechsler Adult Intelligence Scale (WAISIII) and the Wechsler Intelligence Scale for Children (WISCIII) (112).

The NEPSY is used to assess neuropsychological development in the domains of executive function, language, visual-spatial processing, sensorimotor and memory and learning (116). Purposes of the NEPSY include the detection of impairments which may interfere with learning and the investigation of both typical and atypical development. The latter turned the NEPSY into a suitable test for our study aim (116). In this thesis, the scaled scores of six different subtests were combined to form scores on three functional domains: attention/executive function (Tower test), language (Speeded naming and Comprehension of instructions), and memory and learning (Narrative memory, Memory for faces and Memory for names). Advantages of the NEPSY include the suitability of the tests for children aged three to twelve years taking age-related levels of performance into account, the use of a wide, single standardization sample and excellent inter-rater reliability (0.97-0.99) (113,116). The NEPSY correlates moderately with other neuropsychological tests, such as the Benton Neuropsychological Tests (116). Another advantage of the NEPSY is that it uses tasks with which children are often confronted in daily life, such as remembering names or faces. This makes the NEPSY suitable to detect deficits that could interfere with learning in everyday life (113). Verbal memory was further evaluated with the with the word pair subtest of the Children's Memory Scale. The Children's Memory Scale has a moderate to high correlation with other tests evaluating memory function (115).

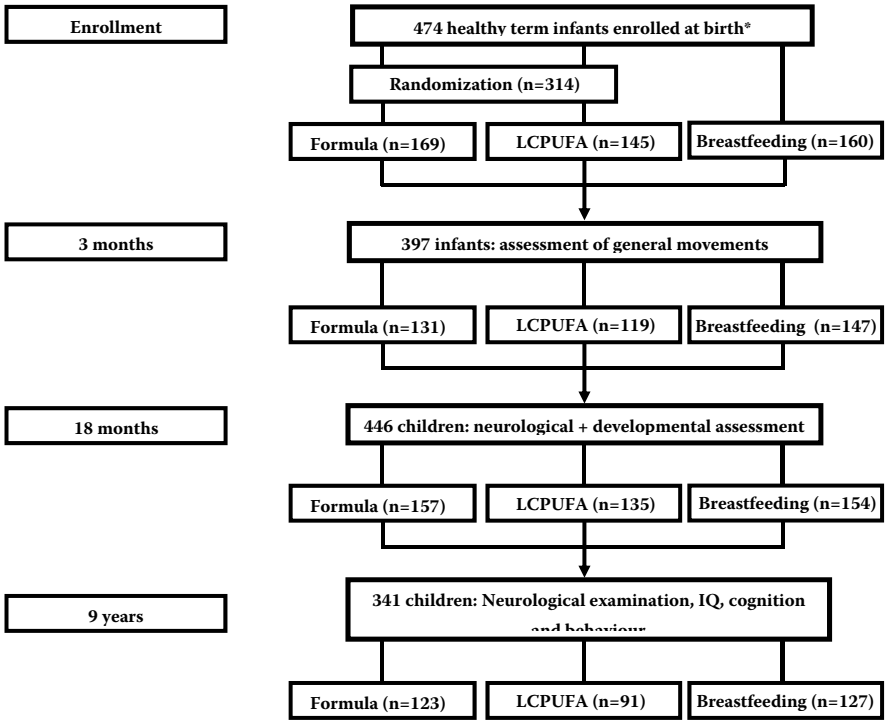
Lastly, attention was evaluated with the TEA-Ch, giving an estimation of three different types of attention: selective attention (Sky Search), sustained attention (Score test and Sky Search Double Task) and attentional switching (Creature Counting test and Opposite Worlds test). Selective attention is the ability to discriminate targets from distractors. When a task does not capture the interest, but maintaining attention is necessary, sustained attention is required. Being able to switch the focus of attention from one task to another is called attentional switching. The TEA-Ch attempts to measure all three types of attention and to provide a pattern of strengths and weaknesses of the child. Although the manual did not specify

a means for calculating measures for the specific types of attention, we calculated scores for selective attention, sustained attention and attentional switching as the average of the various scores belonging to each specific type of attention. In this way we obtained information on the child's performance on a certain type of attention. The TEA-Ch is normed for children between the ages of 6 and 16; by giving norms for six separate age-groups, the test also takes developmental changes of attention into account (114). The TEA-Ch is a standardized and validated test with moderate to good reliability (114,117). As the TEA-Ch does not correlate well with measures of intelligence and general ability, it is a valuable addition to the test battery used in this thesis (117).

As Dutch versions or norms of the WASI, NEPSY, TEA-Ch and CMS were lacking, the tests were translated into Dutch and scores were calculated using the original non-Dutch norms. Although this is not recommended, it is acceptable for the purpose of comparing groups.

Behaviour was assessed in the home and school environment using the Child Behavior Checklist (CBCL) and the Teacher's Report Form (TRF) respectively (118), which gives information about the child's behaviour on nine different problem scales: withdrawn/depressed behaviour, somatic complaints, anxious/depressed behaviour, social problems, thought problems, attention problems, sex problems, delinquent behaviour and aggressive behaviour. Furthermore, two social scales are formed from the problem scales: internalizing behaviour – comprised of the problem scales somatic problems, anxious/depressed and withdrawn/depressed behaviour - and externalizing behaviour - formed by the problem scales delinquent and aggressive behaviour. Reliability and validity of the CBCL and TRF are good (118). Furthermore, the CBCL correlates well with other measures of behaviour, such as the Parent Questionnaire and the Revised Behavior Problem Checklist (0.81-0.82); the correlations for the problem scales are slightly lower, but remain moderate to good (118).

Flow chart of participating children of the Groningen LCPUFA project from study enrolment until 9 years follow-up.



Abbreviations: LCPUFA: long chain polyunsaturated fatty acids.
* Two of the 474 infants met the exclusion criteria and were excluded from analyses.

Table 1. *Criteria for dysfunction per domain (111)*

Cluster	Based on	Criteria for the presence of a deviant cluster
1. Posture and muscle tone	– Posture during sitting, standing and walking – Muscle tone	Two or more of the following: – mild deviations of muscle tone in legs – mild deviations of muscle tone in arms – consistent mild deviations in posture
2. Reflexes	– Intensity tendon reflexes arms: high, low or asymmetrical – Threshold tendon reflexes arms: high, low or asymmetrical – Intensity tendon reflexes legs: high, low or asymmetrical – Threshold tendon reflexes legs: high, low or asymmetrical – Foot–sole response: uni– or bilateral Babinski sign – Plantar grasp: uni– or bilaterally present – Abdominal reflex: asymmetry	Presence of at least two signs
3. Involuntary Movements	– Spontaneous motor behaviour – Test for involuntary movements – Movements of face, eyes, tongue	Presence of at least one of the following: – marked, consistent choreiform movements of distal muscles – marked, consistent choreiform movements of proximal muscles – marked choreiform movements of face, eyes and/or tongue – marked, consistent tremor – consistent athetotiform movements in distal muscles
4. Coordination and balance	– Finger–nose test – Fingertip–touching test – Diadochokinesis – Kicking – Knee–heel test – Reaction to push, sitting – Reaction to push, standing – Romberg test – Walking along a straight line – Standing on one leg – Hopping on one leg	Three or more tests inappropriate for age
5. Fine manipulation	– Finger–opposition test: smoothness – Finger–opposition test: transition – Follow–a–finger test – Circle test	Two or more tests inappropriate for age
6. Associated movements	Associated movements during: – diadochokinesis – finger–opposition test – walking on toes – walking on heels Mouth–opening–finger–spreading– phenomenon	Presence of an excessive amount of associated movements for age in at least three tests
7. Sensory Function	Graphestesia Kinaesthesia Sense of position Hearing Visual fields	Two or more sensory functions dysfunctional
8. Cranial nerve function	Motor behaviour of face, eyes pharynx and tongue	Mild cranial nerve palsy

Outline of the thesis

These two projects offered us the opportunity to examine the association between MND and parental anxiety and well-being as well as the relations between MND and learning and behavioural problems. Three parts can be distinguished in the following section of this thesis:

Part I: parental anxiety and minor neurological dysfunction in infants:
Chapter 2 describes parental anxiety and well-being as an early risk factor for MND approximately one year after birth.

Part II: neurocognitive and neurobehavioural associations at school age:
In chapter 3, the relationship between severity and type of MND and IQ is reported. The specific cognitive impairments underlying this association are described in chapter 4. This is followed by a report of the relation between MND and behavioural problems in chapter 5. Sex differences in the interaction in the neurocognitive and neurobehavioural association will also be addressed in these chapters.

Part III: general discussion and summary.
Chapter 6 consists of a general discussion on the implications and underlying substrate of having MND and of the sex differences in neurocognitive and neurobehavioural associations and future perspectives. Finally, a summary of the results in English as well as Dutch is given in chapter 7 and 8 respectively.

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Part I

Parental anxiety and minor neurological dysfunction in infants

Chapter 2

Maternal anxiety is related to infant neurological condition, paternal anxiety is not

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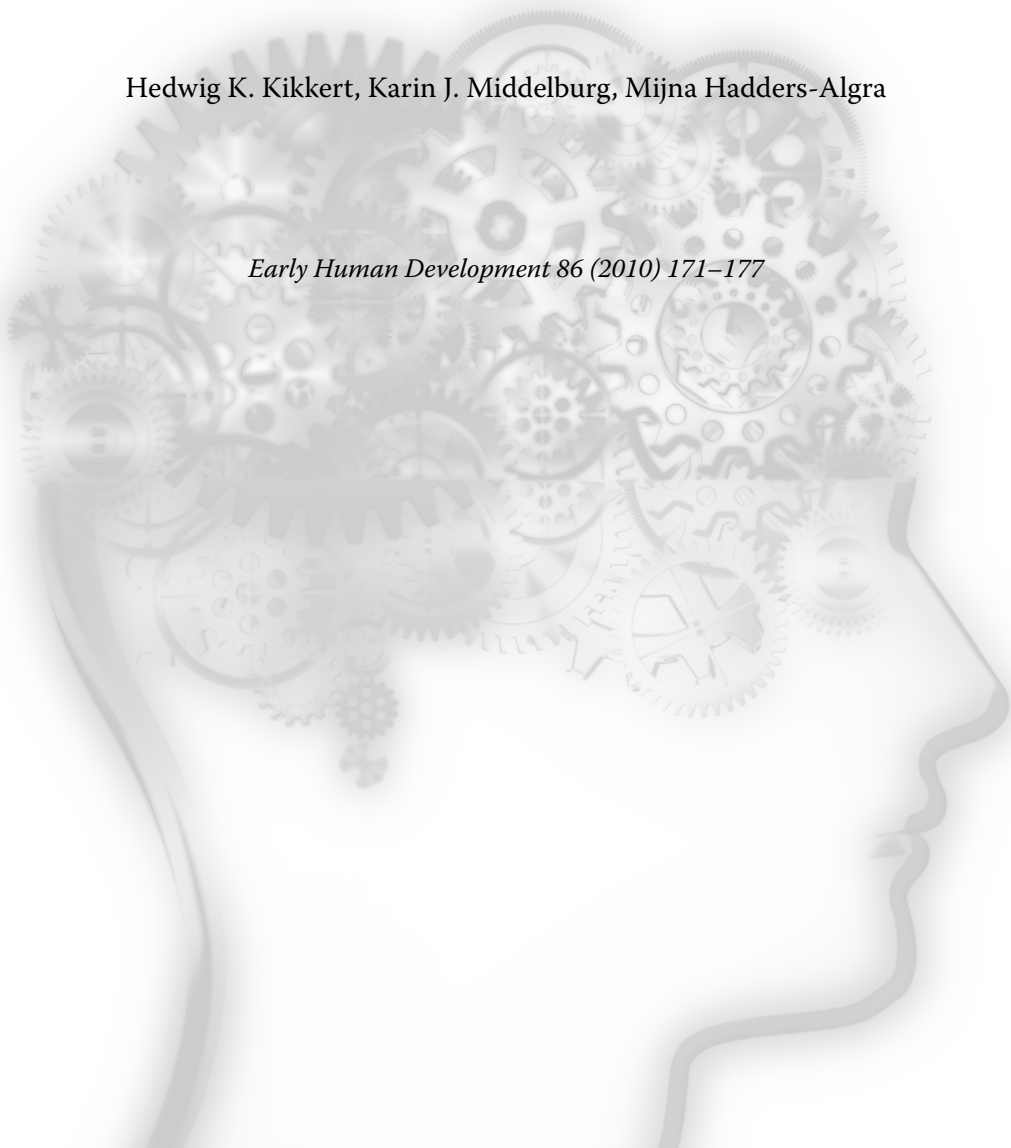

Abstract

Background: Parental anxiety and stress may have consequences for infant neurological development. Aims: To study relationships between parental anxiety or well-being and infant neurological development approximately one year after birth.

Study design: Longitudinal study of a birth cohort of infants born to subfertile couples. Subjects: 206 parent– child dyads.

Outcome measures: Infant neurology was assessed with the Touwen Infant Neurological Examination (TINE) at 10 months and a developmental questionnaire at 12 months. Parental measures included trait anxiety measured by the State-Trait Anxiety Inventory (STAI) and well-being measured by the General Health Questionnaire (GHQ).

Results: Maternal trait anxiety was associated with a less optimal neurological condition (rs=–0.19, pb0.01) of the infant. This association persisted after adjusting for confounders and results were confirmed by the outcome of the developmental questionnaire. Paternal trait anxiety and parental well-being were not related to the infant's neurodevelopmental outcome.

Conclusions: Infants of mothers with high trait anxiety have an increased vulnerability to develop a non- optimal nervous system. The association may be mediated in part by early programming of monoaminergic systems. Future research should include an exploration of specific windows of vulnerability to maternal anxiety.

1. Introduction

Parental anxiety and well-being may affect infant development. This is true for parental anxiety during pregnancy and for depression during the child's early postnatal years (1,2). Aspects of maternal emotional state during pregnancy, such as fear of giving birth, pregnancy hassles and life stress, are associated with lower scores of motor development at 8 months and mental development at 8 and 24 months on the Bayley Scales of Infant Development (BSID) and with difficult behaviour at 3 and 6 months (1,3,4). Indications have been found that the associations may be mediated by high levels of maternal cortisol, as higher cortisol levels were associated with lower BSID scores (1) and more difficult behaviour (5) during infancy. Studies which used the State-Trait Anxiety Inventory (STAI; (6)), suggest similar associations. For example, mild forms of maternal anxiety measured by STAI have been associated with negative infant reactivity towards novel situations (7), difficult temperament at 4 and 6 months (8) and lower mental and motor development at 2–3 days and at 12 and 24 months (9,10). Additionally, anxiety measured by the STAI has been associated with increases in fetal motor activity (11) and fetal heart rate (12). Programming of the hypothalamo–pituitary–adrenal (HPA) axis of the fetus offers an explanation for the above mentioned associations. Possibly, maternal stress hormones influence the HPA axis of the fetus either by direct transport of maternal stress hormones into the fetal circulation or by reducing uteroplacental blood flow and supply of nutrients to the fetus. This could have direct consequences for fetal growth and development or indirect consequences as reduced nutrient and oxygen supply could activate the fetal HPA axis. The resulting higher fetal cortisol may affect early brain development (13).

In addition, the postnatal parental emotional state may also be related to infant development. Stress in parents of 2 year olds was associated with externalizing behaviour of the child as measured by the Child Behavior Checklist (14). Postnatal maternal depression has been related to lower scores on mental and motor development according to the BSID at 15 months of age (2) and to internalizing and externalizing behaviour problems (15). Not only has maternal behaviour been associated with child development during early childhood, paternal behaviour may also affect the child's developmental outcome. Children of fathers with a postnatal depression had more emotional and behavioural problems than children of non-depressed fathers (16).

Parental anxiety has also been associated with altered parent–infant interactions (17,18). The direction of the association is, however, not clear. It might be that parental stress induces altered child behaviour and development, but alternatively it might also be that specific infant behaviour, such as anxiety (19) or the inability to walk (20) influences parent–infant interactions resulting in increased parental anxiety. Additionally, both parties may share a genetically determined sensitivity to stress.

No data exists on the relation between parental anxiety and the infants' neurological condition. The data of the Groningen ART-cohort (Assisted Reproductive Technology; (21)) offered the possibility to study relationships between parental behavioural characteristics one year after child birth (maternal and paternal anxiety and well-being) and the infant's neurological condition in terms of minor neurological dysfunction (MND) and neurological optimality at 10 months. We hypothesize that increased parental anxiety and decreased parental well-being result in a less optimal neurological development of the infant. As infant development has been related to postnatal stress and depression of both the mother and father, the presence of an association between maternal as well as paternal emotional state and infant condition would suggest that postnatal parental behaviour or a shared genome mainly explains the association. Accordingly, if associations with infant neurology are restricted to maternal anxiety this would be an argument in favour of a prenatal, biological effect of anxiety. To validate the significance of neurological findings for daily life a parental questionnaire on motor development was used.

2. Methods

2.1. Subjects

Two hundred and ten parent–child dyads were available to this study, of which 206 participated at 10 months. They were part of a longitudinal study about relationships between assisted reproduction and neurodevelopmental outcome (21–23). The children were born to subfertile couples who were recruited at the department of reproductive medicine of the University Medical Center Groningen and were either conceived naturally (n=90) or by means of IVF (120). Twins and children born from donor-gametes were excluded from the study. Characteristics of the infants and parents can be found in Table 1. At 12 months, 196 parent–child dyads participated.

Participation at 12 months was non-selective: participants and non- participants were similar on demographic variables such as gestational age, birth weight, infant sex, neurological optimality score at 10 months and parental age and education (results not shown). The ethics committee of the University Medical Center Groningen approved the study design and all parents provided written informed consent for participation of their infants in the study.

Table 1. *Descriptive characteristics of infants (n=206) and maternal (n=196) and paternal characteristics (n=192)*

	Participants
Infant sex (male), n (%)	104 (51%)
Breast fed > 6 weeks, n (%)	93 (45%)
Gestational age, mean (SD)	39.6 (1.8)
Birth weight (grams), mean (SD)	3462 (571)
Firstborn, n (%)	133 (65%)
Apgar score at 5 minutes < 7, n (%)	1 (1%)
Infants' corrected age at neurological examination (months), mean (SD)	10.2 (0.4)
Infants' corrected age at follow-up questionnaire (months), mean (SD)	12.3 (0.7)
Maternal age at time of conception of child (years), mean (SD)	32.7 (3.6)
Paternal age at time of conception of child (years), mean (SD)	36.0 (5.1)
Ethnicity mother, n (% Caucasian)	198 (96%)
Ethnicity father, n (% Caucasian)	195 (95%)
Education mother	
Lower, n (%)	19 (9%)
Middle, n (%)	107 (52%)
Higher, n(%)	80 (39%)
Education father	
Lower, n (%)	26 (13%)
Middle, n (%)	100 (49%)
Higher, n(%)	80 (39%)
Smoking (during pregnancy) mother, n (%)	23 (11%)
Alcohol (during pregnancy) mother, n (%)	5 (2%)

3. Instruments

3.1. Neurodevelopmental outcomes

At ten months, infants were examined with the Touwen Infant Neurological Examination (TINE; (24)). Findings of the TINE were classified according to age-specific norms into clusters of dysfunction. Five clusters were distinguished: dysfunctional reaching and grasping, gross motor dysfunction, brain stem dysfunction, visuomotor dysfunction and sensorimotor dysfunction. Children were classified as neurologi-

cally abnormal in case of the presence of a full-blown neurological syndrome, such as a hemisyndrome or a clear hypo- or hypertonia. Infants were classified as MND when more than two clusters fulfilled the criteria for dysfunction. Two forms of normal neurological development were distinguished: normal– suboptimal when one or two clusters fulfilled criteria for dysfunction and neurologically normal when none of the clusters met the criteria for dysfunction. In addition, a neurological optimality score was calculated (25,26). For every item of the assessment the optimal performance was determined (achieved by at least 10% of the infants) and awarded with a point. The number of items fulfilling the criteria for optimality form the neurological optimality score. The maximum score at 10 months is 38 points (see Appendix). We also calculated six subscores: reaching and grasping (5 items), gross motor function (17 items), brain stem function (3 items), visuomotor function (7 items), sensorimotor function (4 items) and language and acoustics (2 items). TINE during the first year of life has a good inter-rater reliability (κ=0.83), sensitivity (91%), and positive and negative predictive power for neurological condition at 18 months (78% and 86%). The specificity of the TINE for outcome at 18 months is moderate (67%) (24).

When the child had reached the age of 12 months, parents were asked to fill out a questionnaire on the development of their child. This developmental questionnaire was adapted from Crowther et al. (27). It consisted of 27 milestone oriented items in the domains of gross motor development, fine motor development and communication. An achieved item was awarded a point, missing items obtained 0.5 points; summation of the points resulted in the total score.

3.2. Parental anxiety and well-being

To assess parental anxiety and well-being 12 months postpartum, a Dutch version of the State-Trait Anxiety Inventory (STAI; (28)), and the Dutch version of the General Health Questionnaire (GHQ; (29)) were used. The STAI measures trait anxiety, which is the relatively stable attribute of people to perceive situations as threatening and thus to react to these situations with increased anxiety. Both parents were asked to consider twenty statements about the way they've been feeling in general over the last 12 months. The questionnaire consisted of positive items, such as: "I am a steady person" and negative items, for example: "I lack self-confidence". Items were scored on a four point Likert scale: 'Almost never', 'sometimes', 'often' and 'almost always', which received respectively a score of 4, 3, 2 or 1 points in case of a positive item and respectively 1, 2, 3 or 4 points in case of a negative item. The resulting score has a minimum of 20 and a maximum of 80 points with a higher score indicating a higher

level of trait anxiety. Missing values were processed according to the manual. This means that questionnaires with 3 or more missing items wereexcluded from the analyses. In case of 1 or 2 missing items, the value '2' was awarded to that question. If a statement was answered with two responses, the response most in line with the other given answers was chosen.

The General Health Questionnaire (GHQ) is also a self-assessment questionnaire. Both parents filled out the Dutch version of the GHQ-30 which consists of 30 questions on well-being. The GHQ is used to identify people with non-psychotic emotional disorders such as anxiety and depression; the total score on the GHQ is an indication of the probability that mental health problems are present and can be seen as the degree of psychological discomfort. With each item, the respondents were asked to compare the way they're feeling at that point in time to the way they feel in general. Items were scored: "not at all", "no more than usual", "rather more than usual" or "much more than usual". Items rated as more or much more frequently than usual were considered as positive (score 1). The addition of the number of positive items resulted in the total score. Missing values were assigned the individual's mean item score. If more than 6 items were unanswered, total scores could not be calculated.

4. Statistical analyses

Statistical analyses were carried out with SPSS, version 14.0. To determine whether attrition bias had occurred, independent t-tests and the Mann–Whitney test were performed to examine differences between the participating families and the families lost to follow-up.

Relationships between parental variables and infant outcomes were evaluated with Spearman's rho. Multivariate statistics were carried out to rule out the influence of possible confounders (multiple regression for neurological optimality scores at 10 months and total developmental score at 12 months; logistic regression for neurological classification at 10 months). Variables were entered into the model if the p-value of the correlation with the outcome measure was smaller than 0.2. Variables that were entered into the regression analysis for neurological classification at 10 months were maternal education, type of feeding (breastfeeding or formula feeding), gestational age, birth weight and Apgar score after 5 min. Adjustment variables for the total developmental score at 12 months were birth weight, gestational age, the time it took to conceive, maternal alcohol use during pregnancy and paternal ethnicity. The level of significance was set at 0.05.

5. Results

At the age of 10 months four infants were classified as MND, 79 as normal–suboptimal and 123 as normal. In the analyses the infants with MND and the infants with a normal–suboptimal neurological condition were pooled into a group which we labelled 'non-optimal neurological condition'. Table 2 shows details on infant neurological condition, the developmental scores at 12 months and data on parental anxiety and well-being. Mean maternal and paternal STAI scores of this study were 31.80 and 28.89. These values correspond to the third decile of the norm population indicating relatively low levels of stress in our study group (28). Mean GHQ scores (Table 2) were also slightly lower than the mean of the normpopulation (29).

Table 2. *Descriptive statistics for outcome variables*

Infant neurological condition at 10 months (n=206)	
Neurological classification	
Normal, n (%)	123 (60%)
Suboptimal, n (%)	79 (38%)
MND, n (%)	4 (2%)
Neurological optimality score, median (range)	29 (18-34)
Reaching and grasping, median (range)	4 (1-5)
Gross motor function, median (range)	12 (5-16)
Brain stem function, median (range)	3 (1-3)
Visuomotor function, median (range)	7 (5-7)
Sensorimotor function, median (range)	3 (0-4)
Language and acoustic NOS, median (range)	1 (0-2)
Infant developmental questionnaire at 12 months (n=196)	
Total developmental score, mean (SD)	19.1 (3.6)
STAI trait anxiety, mean (SD)	
Maternal (n=196)	31.80 (7.89)
Paternal (n=192)	28.89 (6.52)
GHQ, mean (SD)	
Maternal (n=195)	2.97 (4.23)
Paternal (n=191)	2.02 (3.50)

GHQ: general health questionnaire

STAI: State-Trait Anxiety Inventory

5.1. Parental characteristics and neurological condition

Mothers of infants with a non-optimal neurological condition had a significantly higher trait anxiety than mothers of infants with a normal neurological classification (Mann–Whitney; p = 0.02) (Fig. 1a). Paternal trait anxiety (Mann–Whitney; p = 0.32), maternal well-being (Mann–Whitney; p = 0.17) and paternal well-being (Mann–Whitney; p = 0.40) were not related to infant neurological classification (Fig. 1b–d).

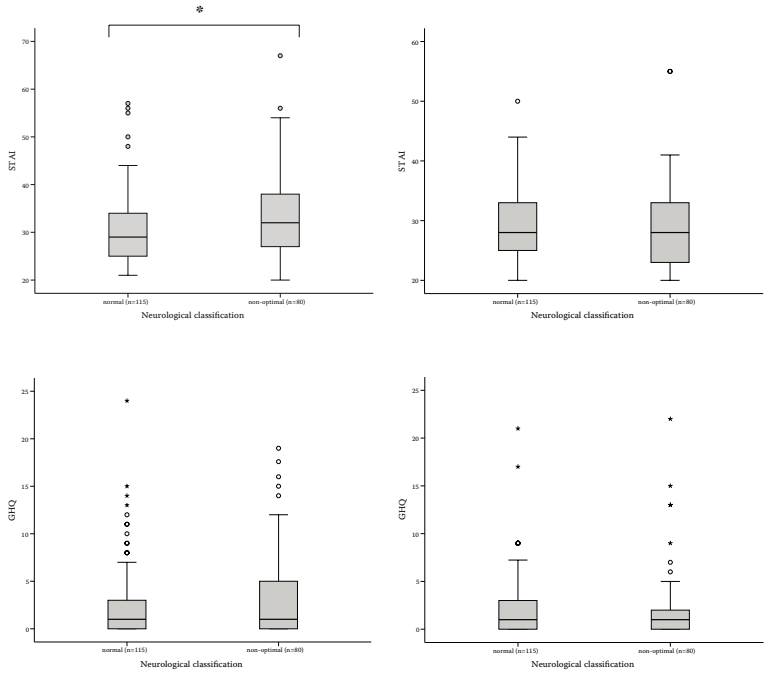

Figure 1. Maternal and paternal trait anxiety and well-being scores and neurological condition at 10 months. Bold horizontal lines indicate median values; the boxes represent interquartile ranges (IQR), the vertical lines 1.5 IQR and the separate points outliers. * $p < 0.05$

Logistic regression analysis (Table 3) revealed that the relationship between maternal trait anxiety and a non-optimal neurological condition at 10 months remained statistically significant after correc- tion for the effect of confounders (OR=1.04; 95% CI=1.00–1.08; p = 0.031).

Table 3. *Best model of logistic regression analyses explaining non-optimal neurological condition at 10 months.*

Factors	OR	95% confidence interval
Maternal trait anxiety	1.04	1.00–1.08
Maternal education	0.53	0.33–0.85
Gestational age	0.82	0.69–0.97

Model explains 13% of variance

Table 4. *Correlations between parental anxiety and well-being and infant neurological optimality scores (NOS).*

	STAI		GHQ-30	
	Maternal	paternal	Maternal	paternal
Neurological optimality score	-0.19**	-0.04	-0.10	-0.10
Reaching and grasping NOS	-0.16*	0.00	-0.10	0.02
Gross motor NOS	-0.14	-0.07	-0.05	-0.12
Brain stem NOS	-0.00	-0.08	0.03	-0.06
Visoumotor NOS	0.01	0.08	0.04	0.10
Sensorimotor NOS	-0.15*	0.01	-0.07	0.00
Language and acoustic NOS	-0.07	-0.05	-0.11	-0.11

* p<0.05 ** p<0.01

NOS: neurological optimality score

Maternal trait anxiety was negatively correlated to the total neurological optimality score at 10 months and to the subscores reaching and grasping and sensorimotor function, indicating that higher maternal trait anxiety was related to lower optimality scores (Table 4). Multiple regression analyses showed that the relationships between maternal anxiety and neurological optimality scores remained statistically significant after correcting for confounders (Table 5). Maternal anxiety was not related to the neurological subscores of brain stem function, gross motor function, visuomotor function and language and acoustics. Paternal trait anxiety, and maternal and paternal feelings of well-being were not related to neurological optimality scores at 10 months (Table 4).

Table 5. *Models of regression analyses explaining variance in neurological optimality scores (NOS).*

Factors	STAI		Maternal education		Birth weight		Explained Variance (%)
	β	p	β	p	β	p	
NOS	-0.20	<0.01	0.19	<0.01	0.16	0.02	9.5
Reaching and grasping NOS	-0.18	0.02	0.17	0.02	-	-	5.0
Sensorimotor NOS	-0.15	0.04	-	-	-	-	1.6

5.2. Developmental questionnaire at 12 months

Results of the developmental questionnaire corroborated the results of the neurological examination at 10 months. The total score of the developmental questionnaire was negatively related to maternal trait anxiety (rs= –0.22, pb0.01) and paternal well-being (rs=–0.17, p=0.02). The associations between maternal anxiety and total developmental score remained statistically significant in the multiple regression analysis (B: – 0.108; 95% CI: – 0.171; – 0.045 p = 0.001). However, the association between paternal well- being and total developmental score disappeared in the multiple regression analysis. Maternal well-being and paternal trait anxiety were not related to outcomes of the questionnaire.

6. Discussion

The present study reported a specific relationship between maternal trait anxiety and infant neurological development which persisted after correcting for confounders. Paternal trait anxiety and maternal and paternal well-being were not related to the infant's neurological condition.

Methodological limitations of this study are the point in time when the parental questionnaires were filled out and the history of subfertility of the parents. With respect to the latter, this means that the results of the present study cannot be generalized. Outcome of the present study was, however, not affected by IVF- treatment (22,23). The fact that the questionnaires were filled out when the child was one year of age, means that we do not have direct measures of parental anxiety and well-being during pregnancy. Furthermore, there was a time interval of 2 months between the infant's neurological examination and the parental questionnaires, which may have influenced the association between the two measures. However, trait anxiety

is a stable measure reflecting a person's tendency to perceive situations as threatening, including pregnancy and child birth (30). Strengths of the current study include the use of a subtle and detailed neurological examination and the validation of the neurological examination with a developmental questionnaire. In addition, both maternal and paternal trait anxieties and well-being have been assessed.

Our results are in line with findings from Huizink et al. (1) and Brouwers et al. (9) who found negative associations between prenatal maternal anxiety and motor development at 3, 8 and 24 months. However, in contrast to the results of Brouwers et al., the association found in the current study remained after adjusting for

confounders. Presumably this difference may be attributed mainly to the larger study sample of the current study. The alternative explanation, i.e. that the association between maternal anxiety during pregnancy and the infant's neuromotor outcome first becomes visible when subtle neurological parameters are used, is refuted by the presence of an association between maternal anxiety and parental reports of infant development. Furthermore, the results are in line with Cornish et al (2), who demonstrated that postnatal maternal chronic depression was related to lower psychomotor development at 15 months. Cornish et al (2) also indicated that brief depression was not related to psychomotor development. This corresponds to our findings that trait anxiety, a stable measure, was related to infant neuromotor development, but momentary well-being was not.

Although maternal anxiety showed a statistically significant association with reduced optimality, the effect-size was relatively small. The effect-size may be influenced by the anxiety scores of the study population: 82% of the mothers had anxiety scores below the mean of the norm population and only 3% scored one standard deviation above the mean of the norm population (28). This may be due to the strict selection of subfertile couples for the IVF programme. Infertility has been associated with negative emotional feelings (31), however, it has been demonstrated that negative emotions associated with infertility disappear when treatment has been successful (32,33). Furthermore, a recent study indicated that a higher number of ART-treatments and a longer time to pregnancy are associated with less anxiety (22). Perhaps couples with less anxiety are more capable of coping with the demanding ART-treatments and as a result selection occurs of couples with low trait anxiety. This is supported by the fact that the main cause of drop-out from ART is emotional distress (34–36). Another possibility is that parents who have had ART feel the need to give social desirable answers, leading to lower scores on self-assessment questionnaires. Additionally, it should be realized that a recent Dutch study on prenatal anxiety in healthy pregnant women reported similar low anxiety scores (37). This may mean that women who participate in studies on pregnancy and development show less anxiety than the general population or that the level of anxiety in pregnant women and women with young children has decreased recently. Nevertheless, ma-

ternal anxiety was not only related to the neurological optimality score, but also to the outcome of the developmental questionnaire, indicating that anxious mothers perceived their infant's motor development as more delayed than less anxious mothers perceived their child's development. Furthermore, our results concur with previous studies and consequently help to gain knowledge and strengthen evidence on the influence of maternal anxiety on infant neurological development.

The relationship between maternal anxiety and the child's developmental outcome may have been brought about by effects occurring during fetal life, during early postnatal life or during both periods. One explanation is the relationship between maternal cortisol and fetal cortisol, norepinephrine, dopamine and serotonine (38). Animal research indicated that prenatal stress is associated with changes in the monoaminergic systems in the cortex, locus coeruleus, striatum, nucleus accumbens and prefrontal cortex (39), areas which are involved in sensorimotor function, movement preparation, movement execution and coordination (40,41). Our findings of less optimal function in sensorimotor function, and reaching and grasping correspond with minor dysfunction in these areas. Further support for a biological relationship comes from studies investigating the relationship between maternal cortisol and development of breastfed infants. Maternal cortisol levels have been related to infant temperament in breastfed infants, but not in formula fed infants (42). In addition, maternal cortisol levels in breastmilk are associated with performance on the Neonatal Behavioral Assessment Scale (43). Consequently, maternal anxiety may not only affect the HPA axis of the infant prior to birth but also postnatally.

A psychological explanation of the association between maternal anxiety and the infant's developmental outcome is the possibility that anxious mothers restrict their children in exploratory motor activities which may affect neurological development, as it is well known that sensory information derived from motor exploration is necessary to select the appropriate motor behaviour and to adapt motor behaviour to the environment. As mothers are often the primary caregivers, it seems plausible that only maternal anxiety is significantly related to the infant's neurological development in this study, and not paternal anxiety. Another possibility of course is that both pre and postnatal factors contribute to a less optimal neurological condition of children of anxious mothers.

Paternal emotional state has also been known to influence child development. Paternal postnatal depression and anxiety disorders have been related to emotional and behavioural problems in children (16,44). The effect has been attributed to changes in father–child interaction. Fathers with major depression are less responsive and less engaged with their child (45). We did not find relation between paternal anxiety and infant neurological outcome, presumably as a result of the relatively healthy psychiatric status of the fathers participating in our study. However, we did find a negative association between paternal well-being and total score of the

developmental questionnaire. This is in line with previous research, reporting that stressed fathers have a more negative perception of their infant (46). The association was weak however and did not persist in the multiple regression analysis, indicating that in a relatively healthy population paternal well-being does not directly affect the infant's developmental outcome. The absence of clear relations between paternal emotional state and anxiety and infant development, decreases the likelihood that genomic sharing between the parent and child is the major factor explaining the association between maternal anxiety and child development. Nevertheless, it is possible that genetic mechanisms in interaction with the environment induce an increased vulnerability for developmental problems. The association between maternal anxiety and less optimal infant development may be the result of a complex chain of interaction between biological and psychological components: a genetic predisposition to anxiety may result in increased levels of cortisol during pregnancy and breastfeeding, which may alter the infant's HPA axis, which in turn may slightly alter infant behaviour, which in turn may promote maternal anxiety.

7. Concluding remarks

The present study suggests that infants of mothers with high trait anxiety may have an increased vulnerability for developing motor problems. The maternal association suggests a biological relation with infant development, which in part may be mediated by programming of the monoaminergic systems (47). This study is one of the few to examine the relation between infant neurological development and both maternal and paternal emotional state. Future research on the association between maternal stress and developmental outcome should include repeated measurements of both prenatal and postnatal maternal anxiety in order to explore timing effects of maternal stress.

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Appendix

Criteria for the 38 items of the neurological optimality score at 10 months as defined by Hadders-Algra et al. (23).

Item	Criteria for optimality at 10 months
Reaching and grasping	
Goal-directed reaching	Can grasp and hold 3 objects
grasping	pincer grasp or inferior pincer grasp
Posture during reaching/grasping	Age adequate
Quality of reaching	Age adequate
Quality of grasping	Age adequate and symmetrical
Gross motor function	
Crawling	Crawls on hands and knees
Rolling over	Can roll over
Sitting	Can sit independently for at least 10 seconds
Sitting up	Can sit up independently
Standing	Can stand independently for a few seconds
Walking	Can walk with help
Tremor	Absent
Motility in supine	Variable and symmetrical
Motility in prone	Variable and symmetrical
Head balance in sitting	Age adequate
ATNR posturing	Absent or variably present
Traction test	Age adequate and symmetrical
Sitting posture	Age adequate and symmetrical
Standing posture	Age adequate and symmetrical
Walking posture	Age adequate and symmetrical
Prone suspension	Age adequate and symmetrical
Vertical suspension	Age adequate and symmetrical
Brainstem function	
Brainstem function	Normal
Doll's eye phenomenon	Age adequate
Moro reaction	Age adequate
Visuomotor function	
Eyes, fixation	Age adequate
Eyes, movements	Conjugated movements
Visual fields	90° and symmetrical

Item	Criteria for optimality at 10 months
Strabism	Absent
Sunset	Absent
Pupillary reactions	Normal
Nystagmus	Absent
Sensorimotor function	
Passive muscle tone	Normal muscle tone, no asymmetry
Active power	Normal power regulation
Reflexes	Normal threshold and intensity; symmetrical
Foot sole response	Age adequate and symmetrical
Language and acoustics	
Speech	Babbling
Acoustic orientation	Acoustic reaction both sides

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Part II

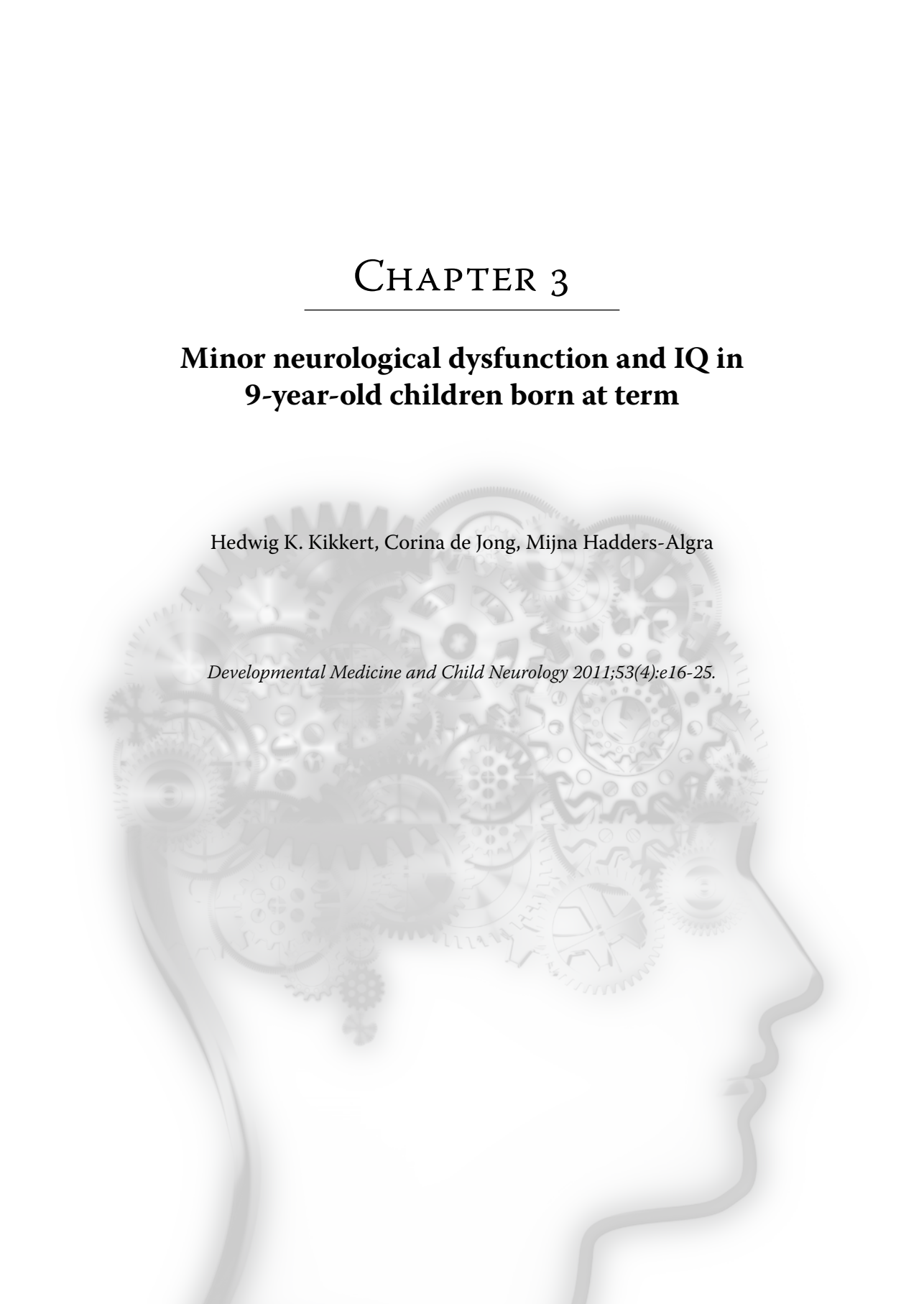
Neurocognitive and neurobehavioural associations at school age

Chapter 3

Minor neurological dysfunction and IQ in 9-year-old children born at term

Hedwig K. Kikkert, Corina de Jong, Mijna Hadders-Algra

Developmental Medicine and Child Neurology 2011;53(4):e16-25.



Abstract

Aim: The aim of this study was to examine the relationship between the severity and type of minor neurological dysfunction (MND) and IQ in 9-year-old children born at term.

Method: Three hundred and forty-one children (177 males, 164 females; mean age 9y, SD 3mo, range 8y 10mo–9y 7mo) who were born at term were neurologically assessed according to Touwen. Children with perinatal risk or with a congenital disorder were excluded. Special attention was paid to the severity and type of MND. Eight domains of dysfunction were distinguished, including fine manipulative ability and coordination. On the basis of the number of dysfunctional domains, the severity of dysfunction was expressed as simple MND (sMND) or complex MND (cMND). Verbal, Performance, and Full-scale IQ (FSIQ) were assessed with the Wechsler Abbreviated Scale of Intelligence. Univariate and multivariate statistical analyses were performed.

Results: Neurologically normal children had higher IQ scores than those with sMND and cMND (mean FSIQ 104 (95% confidence interval (CI) 102–106) to 100 (95% CI 97–102) and 95 (95% CI 91–98) respectively). Multivariate statistics confirmed that the IQ scores of children with sMND and cMND did not differ. Fine manipulative disability and coordination problems were associated with lower IQ scores but other dysfunctions were not.

Interpretation: The type of MND rather than the severity is associated with lower IQ in children born at term.

What this paper adds
- In children born at term, type rather than severity of MND is associated with a lower IQ.
- The results of this study indicate that fine manipulative disability and coordi- nation problems are associated with low IQ.
- This paper suggests that minor dysfunction of the corticostriatothalamocortical and cerebellothalamocortical pathways may be mediators of a low IQ.

1. Introduction

During the last decade the prevalence of developmental disorders, such as attention-deficit–hyperactivity disorder and autism, has increased (1). This increase has led to growing attention being given to minor neurological dysfunctions (MNDs), defined as the occurrence of neurological dysfunction in the absence of apparent neurological pathology, such as cerebral palsy.

Two types of MND may be distinguished: simple MND (s-MND) and complex MND (c-MND). s-MND is only weakly related to perinatal risk factors, whereas c-MND has strong relationships with pre- and perinatal adversities. c-MND is also more clearly associated with learning and behavioural problems than the simple form. Learning problems that are known to be associated with the severity of MND are impairments in reading, spelling, and arithmetic skills, as well as school failure (2-5). Based on the difference in aetiology and the differential association with learning and behavioural problems, it has been hypothesized that s-MND represents a typical but non-optimal form of brain development. In other words, the s-MND denotes minor neurological difference, not dysfunction. On the other hand, complex MND reflects clinically relevant dysfunction of the brain (4). The type as well as the severity of the MND may have clinical significance. Fine manipulative disability, problems in coordination, and – to a lesser extent – choreiform dyskinesia, and dysfunctional posture and muscle tone have been associated with a poorer performance in mathematics, reading, and spelling (5). Another study reported associations between fine manipulative disability and school failure, poorer arithmetic skills, language comprehension, and spelling, and also between coordination problems and school failure (6).

Little is known about the relationship between MND and IQ. Previous studies have indicated that the presence of neurological soft signs is associated with lower IQ scores (7), but details of this relationship are not known. The Groningen Long-chain Polyunsaturated Fatty Acids (LCPUFA) Project offered the possibility of studying the relationships between MND and cognition in healthy 9-year-old children born at term. Based on the aforementioned studies, we hypothesized that the presence and specifically the severity of MND is associated with lower IQ scores. In addition, we expected that problems in the domains of fine manipulative ability and coordination, particularly, are related to lower IQ scores. Specific attention was paid to differences between the sexes. Males are more often diagnosed with developmental disorders, such as attention-deficit–hyperactivity disorder (8) and, in addition, males have a higher prevalence of MND (3). Furthermore, volumetric magnetic resonance imaging studies demonstrated differences between the sexes in a number of areas in the brain, such as a disproportionately larger caudate nucleus and globus pallidus in females and a larger cerebellum in males (9). The differences between males and females in brain maturation may be reflected in differential associations between

neurological development and IQ. Finally, we also investigated whether discrepancies between Performance IQ (PIQ) and Verbal IQ (VIQ) were related to neurological conditions, as others have reported that this may be the case (10).

2. Method

2.1 Participants

The study comprised 341 children (177 males, 164 females) whose ages ranged from 8 years 10 months to 9 years 7 months (mean 9 years, SD 3 months). The children participated in a double-blind randomized controlled trial on the effects of the supplementation of formula with LCPUFA during the first 2 months after birth. At birth, 474 infants born between 37 and 42 weeks of gestation were enrolled in the study. The participants were recruited from hospitals and midwife clinics in and near Groningen, the Netherlands. Children with perinatal risk, for instance infants from multiple births or children with hypoxic-ischaemic encephalopathy, and infants with a congenital disorder were excluded from the study (11). Two groups of infants were randomized into a group receiving formula with LCPUFA (Nutrilon Premium; (Nutricia, Zoeterneer, the Netherlands) with 0.45% (by weight) arachidonic acid and 0.30% (by weight) docosahexaenoic acid; n=145) and a group receiving control formula without LCPUFA (n=169). A third group consisted of infants of mothers who chose to breastfeed their child (n=160). Of the original cohort, 72% participated in the follow-up assessment at 9 years of age (Fig. 1) (11,12).

Extensive information on social background, obstetric conditions, and pre- and perinatal circumstances was collected at birth. This enabled us to form an obstetric optimality score (13). In addition, social background at 18 months was evaluated by the Home Observation for Measurement of the Environment and an abbreviated version of the Wechsler Adult Intelligence Scale (WAIS III) was administered to estimate maternal VIQ.

At 9 years, data on current social situation were collected. Characteristics of the participating children, their parents, and of families lost to follow-up can be found in Table 1. The ethics committee of the University Medical Centre Groningen approved the study design and all parents provided written informed consent for their child's participation in the study.

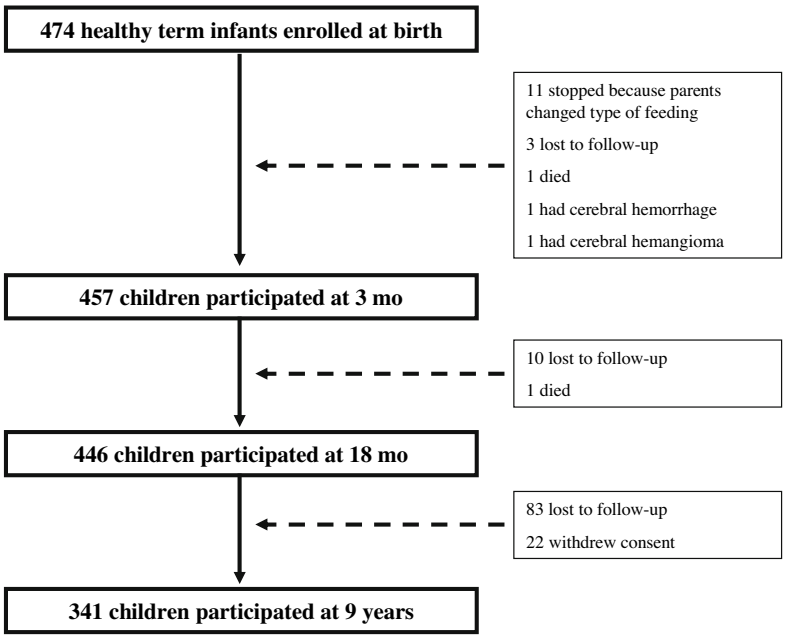


Figure 1. Flow chart of children from study enrolment until 9-years follow-up.

Table 1. *Demographic characteristics of participants (n=341) and their parents*

	Participants		Children lost to follow up	
	Boys (n=177)	**Girls (n=164)**	**Boys (n=78)**	**Girls (n=55)**
% of original study group	69%	75%	31%	25%
Age (years), mean (SD)	9.0 (0.22)	9.0 (0.22)	-	-
Birth weight (g), mean (SD)	3570 (497)	3515 (427)	3573 (405)	3450 (463)
Firstborn, n (%)	68 (40%)	77 (49%)	31 (40%)	21 (38%)
Apgar score at 3 minutes, median (range)	10 (5-10)	10 (7-10)	10 (7-10)	10 (9-10)
HOME, median (range)	43 (33-45)	44 (32-45)	42 (34-44)	42 (38-44)
OOS median (range)	59 (43-69)	59 (43-69)	58 (50-68)	61 (47-67)
Special education, n (%)	13 (7%)	6 (4%)	-	-
Type of feeding after birth*				
Breastfed, n (%)	64 (36%)	63 (38%)	15 (19%)	17 (31%)
LCPUFA supplemented formula, n (%)	42 (24%)	49 (30%)	36 (46%)	19 (35%)
Standard formula, n (%)	71 (40%)	52 (32%)	27 (35%)	19 (35%)
Maternal education				
High (university education or vocational college), n (%)	46 (27%)	42 (27%)	10 (13%)	8 (15%)
Medium (college graduate or junior vocational college), n (%)	101 (59%)	93 (59%)	45 (58%)	25 (42%)
Low (no education or primary education), n (%)	23 (14%)	23 (15%)	23 (30%)	22 (40%)
Paternal education				
High (university education or vocational college), n (%)	51 (32%)	53 (33%)	10 (13%)	13 (24%)
Medium (college graduate or junior vocational college), n (%)	70 (43%)	73 (46%)	35 (46%)	23 (38%)
Low (no education or primary education), n (%)	41 (25%)	34 (21%)	31 (41%)	19 (35%)
Maternal age at conception (years), mean (SD)	30.3 (4.2)	30.2 (4.4)	30.0 (4.9)	29.4 (4.9)
Maternal smoking during pregnancy (>5 cigarettes/day), n (%)	35 (20%)	25 (15%)	14 (18%)	11 (20%)
Maternal alcohol during pregnancy, n (%)	19 (11%)	26 (16%)	7 (9%)	5 (9%)
Maternal hypertension during pregnancy, n (%)	27 (15%)	21 (13%)	8 (10%)	6 (11%)

HOME: Home Observation for Measurement of the Environment

OOS: obstetrical Optimality Score

LCPUFA: Long-chain polyunsaturated fatty acids

* significant difference between participants and children lost to follow up. χ^2 11.70; p=0.003

2.2 Procedures

Neurological condition was assessed with a technique designed for the evaluation of MND (14,15). The Touwen assessment, which was used for this study, is an age-specific examination that takes developmental changes into account. Items of the assessment are grouped into eight domains of dysfunction: posture and muscle tone, reflexes, choreiform dyskinesia, coordination, fine manipulative ability, associated movements, sensory deficits, and cranial nerve function. The criteria for a dysfunctional domain are met when multiple signs of dysfunction are present (16). A distinction between two types of MND is made on the basis of the number of dysfunctional domains: three or more dysfunctional domains signifies c-MND; s-MND is defined as one or two dysfunctional domains (4). In the absence of a dysfunctional domain or in the case of isolated presence of dysfunctional reflexes, the child is classified as neurologically normal. Interrater, intrarater, and test–retest reliability for neurological classification varied from good to excellent (16).

Intelligence was assessed with the Wechsler Abbreviated Scale of Intelligence (WASI). The WASI is a validated screening test that is used to measure various types of intelligence: verbal knowledge, visual information processing, spatial and nonverbal reasoning, and general intelligence (17). Three IQ scores can be formed using the WASI: a Verbal IQ (VIQ) score (subtests: vocabulary and similarities); a Performance IQ (PIQ) score (subtests: block design and matrices), which result in a Full-Scale IQ (FSIQ) score. One of the subtests for PIQ, block design, is in part dependent on motor function. The WASI correlates well with more comprehensive intelligence tests, such as the Wechsler Adult Intelligence Scale (WAIS- III) and the Wechsler Intelligence Scale for Children (WISC- III) (17).

Furthermore, an IQ discrepancy score was calculated by subtracting PIQ from VIQ. Thus, a positive IQ discrepancy score indicates that VIQ was higher than PIQ and a negative score indicates that PIQ was higher than VIQ. An IQ discrepancy score larger than 22 points was considered to be clinically significant (17).

The Touwen assessment and the WASI were carried out by two psychologists (HKK, CdJ). The WASI was scored by the psychologists before the Touwen assessment was evaluated, implying that the psychologists were not blind to results of the WASI during the Touwen examination. MH-A, who was unaware of the WASI results, reassessed all neurological assessments on the basis of video recordings of the assessment. In case of disagreement between HKK, CdJ, and MH-A, results were discussed until a consensus was reached.

2.3 Statistical analysis

The study was not designed to detect differences in IQ. Nevertheless, power analysis based on FSIQ was performed to determine the required sample size needed to detect a difference in IQ scores of at least half an SD (mean 100; SD 15) with 80% power and an alpha of 0.05. Taking into account the proportion of the prevalence of s-MND and c-MND (s-MND: 15%; c-MND: 6% (4)), at least 45 children with c-MND and 111 children with s-MND had to be included in the study.

Analyses were carried out in three steps. First, univariate analyses using one-way analysis of variance (ANOVA) were carried out to establish whether differences in IQ scores existed between children with different neurological classifications. Contrasts were used to examine differences between specific groups. The Student's t-test was performed to examine differences in IQ between children with or without a specific type of dysfunction. χ^2 tests were performed to examine differences in distribution of clinically significant IQ discrepancies among neurologically normal children, children with s-MND and children with c-MND, and children with and without specific types of dysfunction.

Second, multivariate analyses were performed to adjust for the possible influence of both type of feeding after birth and sex. Lastly, additional multivariate analyses were carried out to investigate whether results would be affected when supplementary covariates were taken into account. Variables were entered into the model if the association with either the IQ scores or the neurological classification reached a p value of less than 0.05. Accordingly, the following variables were entered into the regression analyses: maternal VIQ, maternal and paternal education, maternal pre-pregnancy body mass index, maternal age at the beginning of pregnancy, maternal smoking, alcohol and drug use during pregnancy, maternal hypertension during pregnancy, obstetric optimality score, Home Observation for Measurement of the Environment score, birthweight, and Apgar scores at 1 and 3 minutes. The level of significance was set at 0.05. Statistical analyses were carried out with SPSS, version 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1 Severity of minor neurological dysfunction

At the age of 9 years, 45 children (13%) were classified as having c-MND, 126 (37%) as having s-MND, and 170 (50%) as being neurologically normal. The prevalence of MND was higher in males than in females: s-MND was present in 76 (41%) males and 50 (30%) females, and c-MND was present in 31 (17%) males and 15 (8%) females (χ^2; $p<0.001$).

Children with c-MND had a significantly lower FIQ, PIQ, and VIQ than children who were classified as neurologically normal (FSIQ c-MND: mean 95, 95%

CI 91–98; FSIQ neuro- logically normal: mean 104, 95% CI 102–106; PIQ c-MND: mean 95, 95% CI 92–99; PIQ neurologically normal: mean 105, 95% CI 103–107; VIQ c-MND: mean 95, 95% CI 92–99; VIQ neurologically normal: mean 102, 95% CI 100–104) and a lower FSIQ than children with s-MND (analysis of variance; FSIQ: p=0.027). The FSIQ and PIQ of children with s-MND, but not their VIQ, was significantly lower than that of neurologically normal children (FSIQ s-MND: mean 100, 95% CI 97–102; PIQ s-MND: mean 100; 95% CI 98–103; VIQ s-MND: mean 99, 95% CI 97–101; Fig. 2a). Both subtests of PIQ (block design, which in part depends on motor abilities, and matrix reasoning, which does not depend on motor condition) were similarly correlated to neurological classification (block design rs= -0.18; p=0.001; matrix reasoning rs= -0.20; p<0.001).

Next we analysed relationships for males and females separately. Males with c-MND had a significantly lower FSIQ, PIQ, and VIQ than males with s-MND (FSIQ, c-MND: mean 94, 95% CI 90–98; FSIQ, s-MND: mean 102, 95% CI 98–105; PIQ, c-MND: mean 95, 95% CI 90–99; PIQ, s-MND: mean 102, 95% CI 98–106; VIQ, c-MND: mean 95, 95% CI 90–99; VIQ, s-MND: mean 102, 95% CI 99–104) and neurologically normal males (FSIQ: mean 104, 95% CI 101–107; PIQ: mean 105, 95% CI 102–109; VIQ: mean 102, 95% CI 99–105). The IQ scores of males with s-MND did not differ from those of neurologically normal males (Fig. 2b). The FSIQ, PIQ, and VIQ of females with s-MND, however, differed significantly from the IQ scores of neurologically normal females but did not differ significantly from females with c-MND (FSIQ, neurologically normal: mean 103, 95% CI 101–106; FSIQ, s-MND: mean 97, 95% CI 93–100; FSIQ, c-MND: mean 96, 95% CI 90–103; PIQ, neurologically normal: mean 105, 95% CI 102–108; PIQ, s-MND: mean 99, 95% CI 95–103; PIQ, c-MND: mean 98, 95% CI 90–105; VIQ, neurologically normal: mean 101, 95% CI 99–104; VIQ, s-MND: mean 95, 95% CI 92–99; VIQ, c-MND: mean 97, 95% CI 91–103; Fig. 2c). In addition, only FSIQ was significantly lower in females with c-MND than in neurologically normal females.

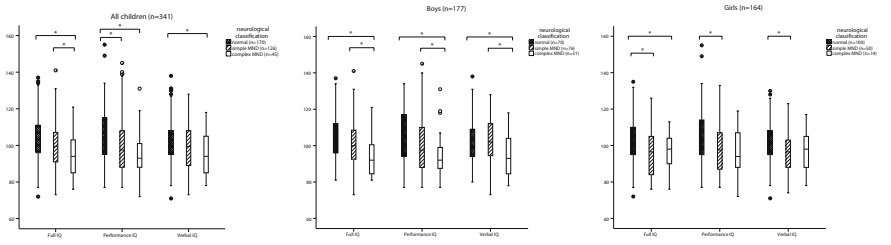


Figure 2. Full-scale IQ, Performance IQ, and Verbal IQ and neurological classification. Bold horizontal lines indicate median values; boxes represent inter- quartile ranges (IQRs); vertical lines represent 1.5 IQR and separate points outliers.*p< 0.05. MND, minor neurological dysfunction.

Multivariate analyses adjusting for both the influence of type of feeding after birth and sex revealed that the occurrence of s-MND or c-MND was significantly associated with lower FSIQ (s-MND: B -4.10, 95% CI -6.94 to -1.26, p=0.005; c-MND: B -8.43, 95% CI -12.49 to -4.37; p=0.001), PIQ, and VIQ (Table 2). Children with c-MND also differed from children with s-MND with respect to FSIQ (B -4.33; 95% CI -8.47 to -0.19; p=0.041). Sex did not contribute significantly to the model for FSIQ (B -2.04; 95% CI -4.68 to 0.61; p=0.131) and PIQ (B -1.06; 95% CI -4.29 to 2.17; p=0.519). With regard to VIQ, a small difference between the sexes was detected, with females performing slightly worse than males (female vs male: B -2.55; 95% CI -5.07 to -0.02; p=0.048). However, no significant interaction was present between sex and neurological classification. When additional covariates were taken into account, the difference in FSIQ between children with c-MND and children with s-MND lost statistical significance. The other associations between neurological condition and IQ were not affected by adjustment for additional covariates.

Table 2. *Multiple linear regression analyses for (a) Performance IQ and (b) Verbal IQ (VIQ), neurological classification, and coordination problem*

	Severity of MND: model parameters						**Coordination problems: model parameters**				
	n	R^2	Effect	95% CI	p-value		*n*	R^2	Effect	95% CI	p-value
(a) Performance IQ											
Step 2: Type of feeding & gender	341	0.15				Step 2: Type of feeding & gender	341	0.08			
Simple MND			-4.43	-7.89;-0.96	0.012	Coordination problems present vs absent			-5.43	-9.04;-1.82	0.003
Complex MND			-8.82	-13.78;-3.87	0.001						
Type of feeding						Type of feeding					
LF vs CF (ref)			-0.50	-4.53;-3.52	0.806	LF vs CF (ref)			-0.53	-4.57;3.52	0.799
BF vs CF			7.00	3.31;10.68	<0.001	BF vs CF			7.31	3.61;11.00	<0.001
BF vs LF			7.50	3.51;11.49	<0.001	BF vs LF			7.83	3.82;11.84	<0.001
Female vs male			-1.06	-4.29;2.17	0.519	Female vs male			-0.60	-3.82;2.63	0.716
Step 3: additional covariates	312	0.18				Step 3: additional covariates	312	0.17			
Simple MND			-3.10	-6.53;0.32	0.075	Coordination problems present vs absent			-3.85	-7.39; -0.31	0.033
Complex MND			-5.99	-10.94;-1.03	0.018						
Type of feeding						Type of feeding					
LF vs CF (ref)			-2.94	-6.96;1.08	0.151	LF vs CF (ref)			-3.08	-7.10;0.94	0.131
BF vs CF			3.12	-0.88;7.12	0.126	BF vs CF			3.19	-0.82;7.19	0.118
BF vs LF			6.06	1.92;10.20	0.004	BF vs LF			6.27	2.13;10.41	0.003
Maternal VIQ			0.22	0.10;0.33	<0.001	Maternal VIQ			0.21	0.10;0.33	<0.001
Maternal prepregnancy BMI			-4.31	-7.47;-1.14	0.008	Maternal prepregnancy BMI			-4.56	-7.72;-1.39	0.005

	Severity of MND: model parameters						Coordination problems: model parameters				
	n	R^2	Effect	95% CI	p-value		*n*	R^2	Effect	95% CI	p-value
Maternal smoking during pregnancy			-5.17	-9.51;-0.84	0.020	Maternal smoking during pregnancy			-5.76	-10.03;-1.48	0.008
(b) Verbal IQ											
Step 2: Type of feeding & gender	341	0.13				Step 2: Type of feeding & gender	341	0.12			
Simple MND			-2.89	-5.60;-0.18	0.036	Coordination problems present vs absent			-3.57	-6.39;-0.75	0.013
Complex MND			-6.09	-9.96;-2.21	0.002						
Type of feeding						Type of feeding					
LF vs CF (ref)			-1.40	-4.55;1.75	0.382	LF vs CF (ref)			-1.41	-4.57;1.76	0.382
BF vs CF			6.92	4.04;9.80	<0.001	BF vs CF			7.14	4.25;10.03	<0.001
BF vs LF			8.32	5.20;11.44	<0.001	BF vs LF			8.55	5.42;11.68	<0.001
Female vs male			-2.55	-5.07;-0.019	0.048	Female vs male			-2.22	-4.74;0.30	0.084
Step 3: additional covariates	285	0.30				Step 3: additional covariates	285	0.28			
Simple MND			-3.91	-6.60;-1.23	0.004	Coordination problems present vs absent			-3.11	-5.89;-0.34	0.028
Complex MND			-4.70	-8.49;-0.92	0.015						
Type of feeding						Type of feeding					
LF vs CF (ref)			-0.98	-4.05;2.09	0.530	LF vs CF (ref)			-1.31	-4.39;1.77	0.402
BF vs CF			3.13	0.03;6.22	0.048	BF vs CF			3.05	-0.07;6.18	0.055
BF vs LF			4.11	0.85;7.37	0.014	BF vs LF			4.37	1.09;7.65	0.009
Maternal VIQ			0.26	0.17;0.36	<0.001	Maternal VIQ			0.26	0.17;0.36	<0.001
Paternal education (middle/high vs low)			5.11	2.11;8.11	0.001	Paternal education (middle/high vs low)			5.08	2.05;8.11	0.001
Gender: female vs male			-4.37	-6.90;-1.85	0.001	Gender: female vs male			-3.87	-6.39;-1.36	0.003
Apgar score at 3 min			2.08	0.40;3.75	0.015	Apgar score at 3 min			2.07	0.38;3.76	0.016

BF / LF / CF: Breastfed / LCPUFA supplemented / Control formula group

Maternal prepregnancy BMI dichotomized as normal vs underweight/overweight; Maternal smoking during pregnancy dichotomized as 0-5 cigarettes/day vs >5 cigarettes/day.

Maternal Verbal IQ: n=312; (no statistically significant difference in maternal education between cases with available information on maternal VIQ and missing data for maternal VIQ: χ^2=1.313; p=0.567)

Paternal education: n= 325; (no statistically significant difference in paternal education at 18 months between cases with available information on paternal education at 9 years and missing data: χ^2= 3.456; p=0.063. If data on paternal education was missing at 9 years, this was substituted by paternal education at 18 months)

Apgar score: n=311; (no statistically significant difference in obstetric optimality score between cases with available information on Apgar score and missing data: Mann-Whitney U=4411.50; p=0.622)

3.2 Type of minor neurological dysfunction

The most frequently occurring dysfunction was of the domain reflexes (n=159; 47%). Dysfunctional coordination was present in 93 (27%) children, 86 (25%) children showed fine manipulative disability, 60 (18%) children had dysfunctional posture and muscle tone, 13 (4%) children had choreiform dyskinesia, and 7 (2%) children had an excess of associated movements. Sensory deficits and cranial nerve dysfunction were present in one child.

Two specific domains of dysfunction were related to lower PIQ and VIQ scores: fine manipulative disability (PIQ, no dysfunction: mean 104, 95% CI 102–106; PIQ, dysfunction: mean 97, 95% CI 94–100; VIQ, no dysfunction: mean 102, 95% CI 100–103; VIQ, dysfunction: mean 95, 95% CI 92–97) and coordination problems (PIQ, no dysfunction: mean 104, 95% CI 102–106; PIQ, dysfunction: mean 98, 95% CI 95–101; VIQ, no dysfunction: mean 101, 95% CI 99–102; VIQ, dysfunction: mean 97, 95% CI 95–100). In males, fine manipulative disability was associated with lower PIQ and VIQ (PIQ, no dysfunction: mean 105, 95% CI 102–108; PIQ, dysfunction: mean 95, 95% CI 91–98; VIQ, no dysfunction: mean 103, 95% CI 101–106; VIQ, dysfunction: mean 95, 95% CI 92–98); however, in females only VIQ was related to dysfunction of the domain fine manipulative ability (VIQ, no dysfunction: mean 100, 95% CI 98–102; VIQ, dysfunction: mean 95, 95% CI 91–100; Fig. 3). Dysfunctional coordination was associated with lower PIQ and VIQ in females (PIQ, no dysfunction: mean 104, 95% CI 101–106; PIQ, dysfunction: mean 97; 95% CI 92–102; VIQ, no dysfunction: mean 100, 95% CI 98–102; VIQ, dysfunction: mean 95, 95% CI 90–99), but in males was associated only with lower PIQ (PIQ, no dysfunction: mean 104, 95% CI 101–107; PIQ, dysfunction: mean 98; 95% CI 94–103; Fig. 3).

Multivariate analyses that were adjusted for the type of feeding demonstrated a statistically significant interaction between sex and fine manipulative ability for PIQ. Males with a fine manipulative disability had lower PIQ than males without this dysfunction. However, the IQ scores of females with and without fine manipulative disability did not differ. For VIQ, the interaction between sex and fine manipulative ability did not reach statistical significance (Table 3). The associations between fine manipulative ability and IQ scores persisted after adjusting for additional covariates, but the interaction between sex and fine manipulative ability disappeared (data not shown). Dysfunction of the domain coordi- nation was associated with lower PIQ and VIQ after adjusting for both the type of feeding and sex (Table 2). No interaction was present between sex and the domain coordination. The association between coordination and PIQ remained in multiple regression analysis adjusting for supplementary covariates (Table 2).

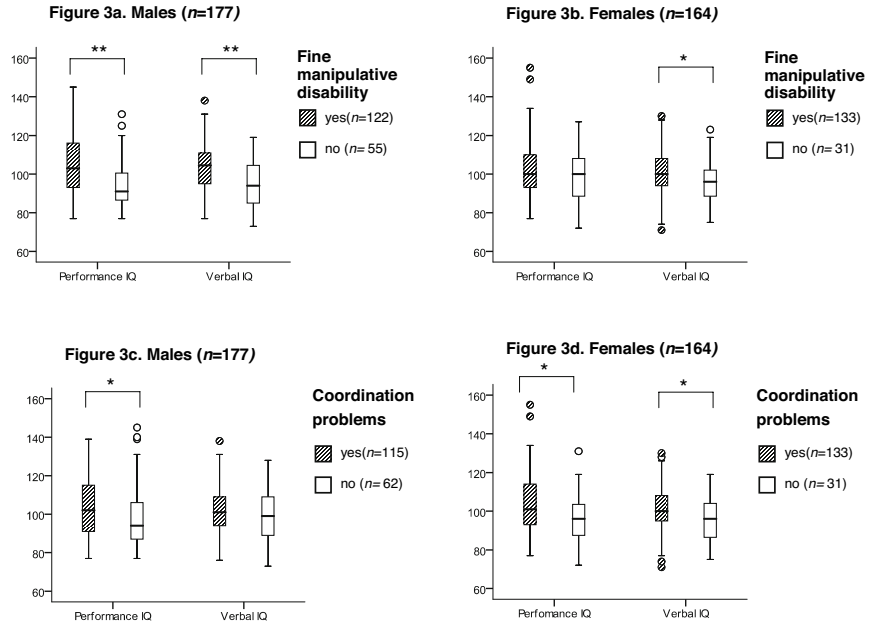


Figure 3. Performance IQ and Verbal IQ and domains fine manipulative ability and coordination for males and females. Bold horizontal lines indicate median values; boxes represent interquartile ranges (IQRs); Vertical lines represent 1.5 IQR and separate points outliers. *p< 0.05; **p< 0.01.

3.3 VIQ-PIQ discrepancy

Correlations between PIQ and VIQ were similar in neurologically normal children, children with s-MND, and children with c-MND (Fig. 4). There was no significant difference in the rate of occurrence of a significant IQ discrepancy among neurologically normal children, children with s-MND, and children with c-MND (χ^2=2.274; p=0.685). Also, the specific domains of dysfunction were not associated with IQ discrepancy (results not shown).

Table 3. *Multiple linear regression analysis: association of fine manipulative disability and IQ scores*

	Performance IQ			Verbal IQ		
	Effect	95% CI	p-value	Effect	95% CI	p-value
Dysfunctional FMA within gender						
Boys: dysfunction vs no dysfunction	-9.31	-14.03;-4.59	<0.001	-7.49	-11.15;-3.83	<0.001
Girls: dysfunction vs no dysfunction	-1.66	-7.47;4.14	0.573	-3.57	-8.06;0.93	0.120
Gender within dysfunctional domain FMA						
Non-dysfunctional FMA: girls vs boys	-2.30	-5.93;1.34	0.215	-3.29	-6.11;-0.47	0.022
Dysfunctional FMA: girls vs boys	5.35	-1.15;11.85	0.106	0.635	-4.40;5.67	0.804
Type of Feeding						
LF vs CF (reference)	-0.42	-4.43;3.60	0.839	-1.34	-4.45;1.77	0.397
BF vs CF	6.79	3.09;10.49	<0.001	6.55	3.68;9.421	<0.001
BF vs LF	7.20	3.20;11.21	<0.001	7.89	4.78;10.99	<0.001

n=341 PIQ: R^2= 0.10 VIQ: R^2= 0.15

FMA: Fine manipulative ability

BF / LF / CF: Breastfed / LCPUFA supplemented / Control formula group

4. Discussion

The present study reported an association between the presence of MND and lower FSIQ, PIQ, and VIQ scores. In particular, two domains of dysfunction contributed to the association: fine manipulative disability and coordination problems.

It is a limitation of the study that participants were part of a randomized controlled trial on the effects of the supplementation of formula with LCPUFA for 2 months after birth. This means that the results of our study cannot be generalized to the whole population of 9-year-old Dutch children. Two specific characteristics of our study group are particularly relevant: the fact that children with perinatal risk were excluded and the fact that only 37% of the children were breastfed after birth, as opposed to 78% in a typical Dutch population (18). Breastfeeding is associated with various social and biopsychological factors including a higher maternal education and a better neurological outcome in 9-year-old children compared with formula feeding (12,19).

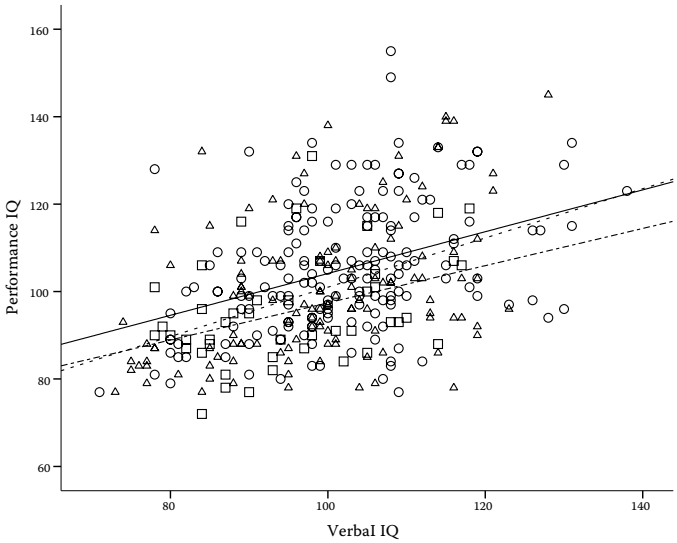


Figure 4. Correlations between Verbal IQ and Performance IQ in neurologically normal children, children with simple minor neurological dysfunction (MND), and children with complex MND. s neurologically normal (n=170); n simple MND (n=126); h complex MND (n=45); neurologically normal, r=0.38, p< 0.001; simple MND, r=0.44, p< 0.001; complex MND, r=0.39; p=0.007.

This may explain the higher prevalence of MNDs in this study, in particular that of s-MND, than in a previous study (4). The high prevalence of both forms of MND may also, in part, reflect the general increase in developmental motor problems (20).

This means that the study population may not be representative of the general Dutch population; however, the resulting larger number of children with s-MND and c-MND offered us the opportunity to study the relationships between the severity of MND and IQ. Multivariate analyses were performed to adjust for the effect of the type of feeding, as we previously had shown that breastfeeding was associated with a lower prevalence of fine manipulative disability (12) and that the type of feeding affected IQ. Breastfeeding was associated with a slight advantageous effect on IQ, and the effect of LCPUFA supplementation depended on the presence or absence of maternal smoking during pregnancy (de Jong et al., personal communication 2010). The exclusion of infants at risk means that our findings cannot be extrapolated to groups of high-risk infants. The notion that neurodevelopmental relationships depend on the type and timing of risk factors operating on the fetal and neonatal brain is supported by studies on the predictive validity of the quality of general movements. The power of the quality of general movements to predict developmental

outcome is considerably lower in the general population than in populations of high-risk infants (21). Despite these limitations, the results of the present study give an insight into associations between the presence of MND and IQ in children at low risk for developmental disorders. Another limitation of the study may be the fact that the psychologists who carried out the neurological assessment were aware of the WASI performance of the children. The bias introduced by this design was however counteracted by the independent evaluation of neurological condition by an assessor who was unaware of WASI results.

Strengths of the current study include the use of a standardized, age-specific detailed neurological examination applied to a relatively large group of children born at term in whom information of many background factors was available.

Our results indicated that the presence of MND was associated with lower IQ scores, which is in line with previous reports on associations between MND and academic achievement (3,5–7). Most probably the association between MND and lower PIQ cannot be attributed to motor dysfunction, as both subtests of PIQ were related similarly to neurological condition. Contrary to our expectations, we did not find associations between the severity of MND and IQ after adjusting for covariates. Three explanations may be offered for the lack of association between the severity of MND and IQ. First, previous studies demonstrating an association between the severity of MND and academic achievement included children who were born preterm or who were small for gestational age (2,3,5), whereas the current study population consisted of children born at term. As mentioned above, associations may differ for children with and without perinatal risk. Second, our study may have lacked the power to demonstrate the effect of severity as our study was only able to detect a difference in IQ scores of more than 7.5 points. A third explanation may be the high prevalence of fine manipulative disability and coordination problems relative to other dysfunctional domains. The high prevalence of these dysfunctions underlines the atypical selection of our healthy term infants, as the prevalences are considerably higher than those in children attending a mainstream school (22). As fine manipulative disability and coordination problems are particularly related to learning problems (5,6), the high prevalence of these dysfunctions may have overruled the relationship between the severity of MND and IQ.

The results indicated that only two domains of dysfunction, fine manipulative disability and coordination problems, were associated with lower IQ scores. However, the study may have lacked the power to demonstrate differences in IQ scores for other domains of dysfunction. Post-hoc power analyses indicated that, with the current sample sizes, 80% power and alpha set at 0.05, it was possible to detect a difference in IQ of at least 12 points for the domain choreiform dyskinesia, 16.5 points

for the domain associated movements, six points for the domain posture and muscle tone, and 4.5 points for the domain reflexes. Therefore, it is possible that the specific dysfunctions may be associated with smaller differences in IQ scores. This may explain why, contrary to the findings of Batstra et al. (5), we did not find an association between choreiform dyskinesia and IQ. However, the finding that only the domains fine manipulative disability and coordination problems were associated with lower IQ scores is in line with Soorani-Lunsing et al.(6), who demonstrated an association between fine manipulative disability, coordination problems, and school failure. This finding may suggest a contribution from the corticostriatothalamocortical and cerebellothalamocortical pathways (4). Activation of these pathways has been recognized in motor and cognitive functions, albeit in different ways. The corticostriatothalamocortical pathways participate in sequential finger movement tasks, movement control, and tasks involving spatial working memory, planning, and attentional switching (23), whereas the cerebellothalamocortical pathways play a role in the initial phases of motor learning, timing of movements, planning of tasks, language, and set-shifting (24,25). However, it should be recognized that few studies have examined clinico-anatomical correlations using standardized and detailed neurological examinations in children (26). Future research combining imaging techniques with standardized neurological assessments should be carried out.

Interestingly, the data suggest some minor interaction between fine manipulative disability and coordination problems, and sex. The interactions disappeared when all covariates were taken into account, which may indicate that the interactions arose because of other differences between males and females. Nevertheless, the tendency that a lower IQ in females may be associated in particular with coordination problems but in males with fine manipulative disability is intriguing. This is especially so because magnetic resonance imaging studies have demonstrated sex-based differences in the maturation of the networks subserving these specific dysfunctions. Could it be that the relatively larger basal ganglia (27) and the higher number of basal ganglia dopamine receptors (28) of females reduce the chance that fine manipulative disability is associated with cognitive impairment? Similarly, is it conceivable that the larger cerebellum of males weakens the association between coordination problems and a lower IQ? These speculations on sex differences in the neural substrate of IQ require further investigation.

Our data did not reveal an association between an IQ discrepancy and the presence or the type of MND, as opposed to a study by Black (10), who demonstrated that in a hospitalized paediatric population the combination of a high VIQ and a low PIQ was associated with a higher neurological dysfunction index. Our results suggest that in a healthy population IQ discrepancies are not related to neurological condition.

5. Conclusion

The present study demonstrates that in healthy children born at term the presence of MND is associated with lower FSIQ, PIQ, and VIQ. The association is brought about in particular by the association between IQ and the two specific types of dysfunction that represent dysfunction of complex supraspinal circuitries: fine manipulative disability and coordination problems.

Acknowledgements

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Chapter 4

Minor neurological dysfunction and cognition in 9-year-olds born at term

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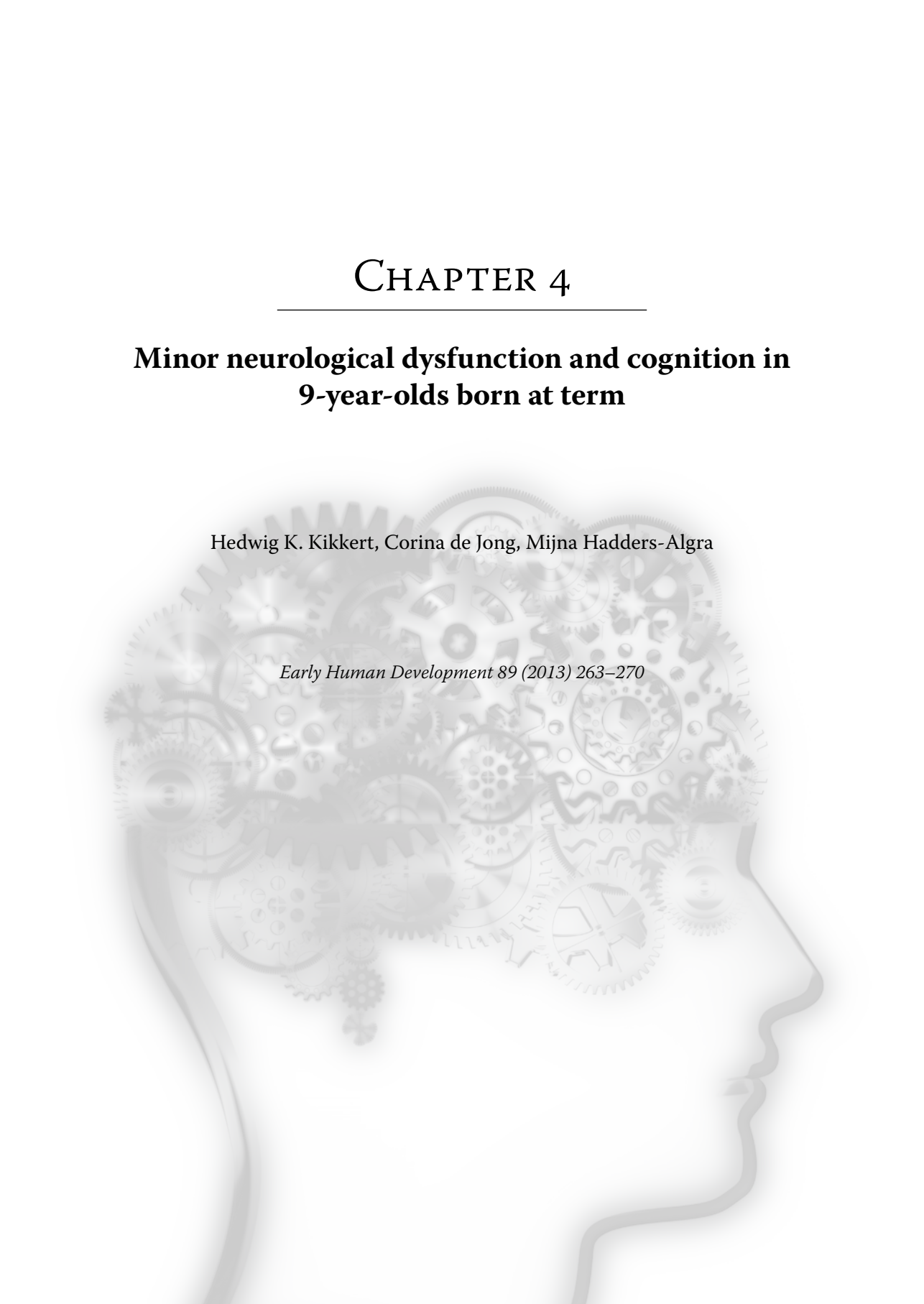
Early Human Development 89 (2013) 263–270



Abstract

Background: In children with developmental disorders, motor problems often co-occur with cognitive difficulties. Associations between specific cognitive deficits underlying learning problems and minor neurological dysfunction (MND) are still unknown.

Aims: To assess associations between specific types of MND as clinical markers of non-optimal brain function and performance in specific cognitive domains.

Study design: Part of a randomized controlled trial.

Subjects: Three hundred and forty one 9-year-old children born at term (177 boys, 164 girls).

Outcome measures: Children were neurologically assessed to detect eight types of MND: mild dysfunction in posture and muscle tone, reflexes, coordination, fine manipulative ability, sensory function, cranial nerve function, choreiform dyskinesia and excessive associated movements. Cognitive function in the domains of attention, memory and language was evaluated using the Test of Everyday Attention for Children (TEA-Ch), a developmental neuropsychological assessment (NEPSY) and the Children's Memory Scale.

Results: Fine manipulative disability and coordination problems were associated with lower scores on attention, memory and learning and language, other types of MND were not. Girls with coordination problems performed significantly worse on attention/executive function than those without this dysfunction; however, in boys, such association was absent.

Conclusion: Particularly, fine manipulative disability and coordination problems were associated with worse cognitive function in the domains of attention, learning and memory and language. Previous and present data suggest a minor sex difference in neurocognitive associations: in girls dysfunction of the cerebello-thalamo-cortical pathways may be associated with cognitive deficits, while in boys cognitive impairment may be associated with dysfunction of cortico-striato-thalamo-cortical pathways.

1. Introduction

Children with developmental disorders frequently exhibit both motor problems and cognitive difficulties. For instance, developmental coordination disorder (DCD) has been associated with attention deficit/hyperactivity disorder (ADHD) and cognitive deficits in the domains of working memory, language and executive functions (1). Children with ADHD often have additional problems with balance, coordination and fine motor skills (1). Also dyslexia has been associated with minor neurological dysfunction (MND), in particular fine manipulative disability (2). In addition, MND has been associated with learning problems, such as problems with reading, spelling and arithmetic (3–5).

MND refers to findings during a standardized and age-specific neurological examination. It denotes the occurrence of minor neurological dysfunction in absence of evident neurological pathology. MND has been associated with perinatal adversities, such as preterm birth (5) and maternal drug or alcohol use during pregnancy (6,7) and can be expressed in severity of MND and in type of MND. The severity of MND is based on a distinction between simple MND and complex MND. Complex MND reflects the clinically relevant form of brain dysfunction on account of its strong associations with a) prenatal and perinatal risk factors, such as gestational age (5), – resembling the etiology of cerebral palsy – and b) learning and behavioral problems. Conversely, simple MND, the more prevalent form of MND, has weak relationships with perinatal adversities and with learning and behavioral problems. Simple MND may be seen as a typical, but non-optimal form of brain development and may in fact be regarded as a minor neurological difference.

The different types of MND include dysfunctional posture and muscle tone, fine manipulative disability and dyscoordination. Specifically, the latter two types of MND have been related to learning problems (4,8) and to lower IQ scores (9). However, the nature of the cognitive deficits underlying these learning difficulties is still unknown. The Groningen LCPUFA (long chain polyunsaturated fatty acids) project offered the possibility to study relationships between MND and specific cognitive domains in nine-year old children born at term. As mentioned above, deficits in memory, attention, executive functions and language have been associated with DCD; DCD in turn is associated with severity and specific types of MND (10). Previously, we reported that in children born at term especially type, rather than severity, of MND was associated with lower IQ (9). Therefore, we aimed to assess relations between specific cognitive domains and type of MND. Based on the above mentioned studies, we expected that specifically fine manipulative disability and coordination problems will be associated with cognitive deficits. In addition, sex differences in the association between MND and cognition were assessed. The rationale for the specific attention to sex was twofold. First, it is well known that sex differences exist in devel-

opmental disorders, for example ADHD occurs more often in boys and eating disorders more often in girls (11). Second, imaging studies have demonstrated differences in the developmental course of the brain (12). Previously, we demonstrated that in boys fine manipulative disability was associated with lower IQ scores, but in girls no such association was found (9). Therefore, we hypothesize that in boys, fine manipulative disability in particular will be associated with lower scores on cognitive tests.

2. Methods

2.1. Participants

Three hundred and forty-one term born children (177 boys, 164 girls) aged from 8 years and 10 months to 9 years and 7 months (mean 9.0 SD 0.22) participated in the study. The children took part in a double-blind randomized controlled trial on the effects of the supplementation of formula with LCPUFA during the first two months after birth. Children with perinatal risk were excluded from the study. Infants were randomized into two groups: a group receiving formula with LCPUFA (Nutrilon Premium® with 0.45% (by wt) arachidonic acid and 0.30% (by wt) docosahexaenoic acid) (n = 145) and a group receiving control formula without LCPUFA (n = 169). A third group of infants were breastfed after birth (n=160). Of the 474 infants enrolled at birth, 72% participated in the follow-up assessment at nine years (Fig. 1; for details see (13)).

Extensive information on social background, obstetric conditions and pre- and perinatal circumstances was collected, which enabled us to form an obstetric optimality score (OOS) (14). The Home Observation for Measurement of the Environment (HOME) was used to evaluate social background at 18 months and an abbreviated version of the Wechsler Adult Intelligence Scale (WAIS III) was applied to estimate maternal verbal IQ.

At nine years, data on current social situation was collected. Characteristics of the participating children and children lost to follow-up can be found in Table 1. The ethics committee of the University Medical Center Groningen approved the study design and all parents provided written informed consent for participation of their child in the study.

Figure 1. Flowchart of children from study enrolment until 9 years follow-up.

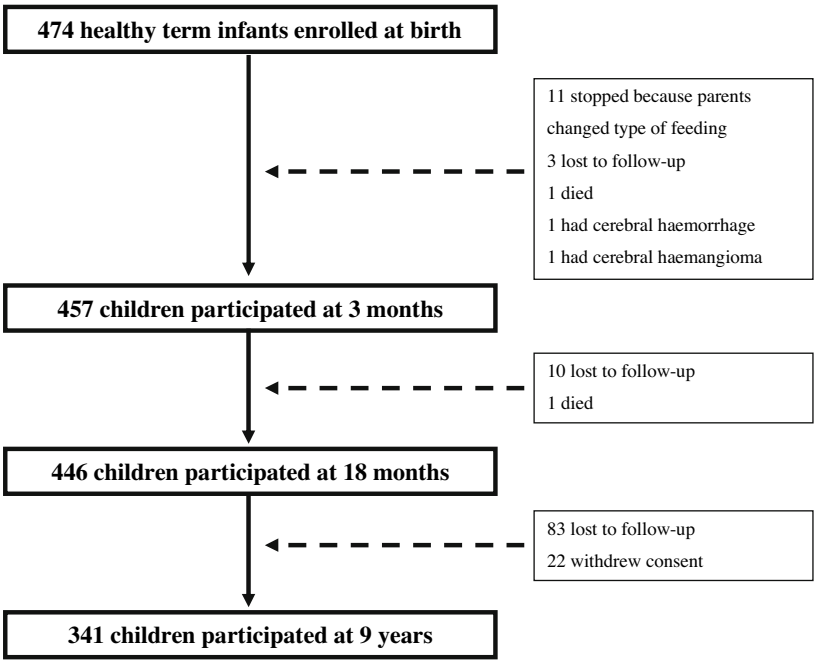


Figure 1. Flowchart of children from study enrolment until 9 years follow-up.

2.2. Procedures

An age-specific technique designed for the evaluation of minor neurological dysfunction (15) was used to assess neurological condition. The assessment, which takes developmental changes into account, was videotaped. Items of the examination are grouped in eight domains of dysfunction: fine manipulative ability, coordination, choreiform dyskinesia, posture and muscle tone, reflexes, associated movements, sensory deficits and cranial nerve function. Presence of multiple related dysfunctional items is required for the classification of a dysfunctional domain (16). Inter-rater, intra-rater and test–retest reliability for neurological classification varied from good to excellent (16).

Cognitive functions were assessed using the Test of Everyday Attention for Children (TEA-Ch) (17), a developmental neuropsychological assessment (NEPSY) (18), and the Children's Memory Scale (19). The TEA-Ch assesses three different types of attention: selective attention (Sky Search test), sustained attention (Score test and Sky Search DT) and attentional switching (Creature Counting test and Opposite Worlds test). Scores of subtests belonging to a specific kind of attention were averaged to give an estimate of that type of attention. The TEA-Ch is a standardized and validated test with age-specific norms and moderate to good reliability (17).

Table 1. *Demographic characteristics of participants (n=341), non-participants (n=133) and their parents*

	Participants		Non-participants	
	Boys (n=177)	**Girls (n=164)**	**Boys (n=78)**	**Girls (n=55)**
% of original study group	69%	75%	31%	25%
Age (years), mean (SD)	9.0 (0.22)	9.0 (0.22)	-	-
Birth weight (g), mean (SD)	3570 (497)	3515 (427)	3573 (405)	3450 (463)
Firstborn, n (%)	68 (40%)	77 (49%)	31 (40%)	21 (38%)
Apgar score at 3 minutes, median (range)	10 (5-10)	10 (7-10)	10 (7-10)	10 (9-10)
HOME, median (range)	43 (33-45)	44 (32-45)		42 (38-44)
OOS, median (range)	59 (43-69)	59 (43-69)	58 (50-68)	61 (47-67)
Special education, n (%)	13 (7%)	6 (4%)	-	-
Type of feeding after birth*				
Breastfed, n (%)	64 (36%)	63 (38%)	15 (19%)	17 (31%)
LCPUFA supplemented formula, n (%)	42 (24%)	49 (30%)	36 (46%)	19 (35%)
Standard formula, n (%)	71 (40%)	52 (32%)	27 (35%)	19 (35%)
Maternal education				
High (university education or vocational college), n (%)	46 (27%)	42 (27%)	10 (13%)	8 (15%)
Medium (college graduate or junior vocational college), n (%)	101 (59%)	93 (59%)	45 (58%)	25 (42%)
Low (no education or primary education), n (%)	23 (14%)	23 (15%)	23 (30%)	22 (40%)
Paternal education#				
High (university education or vocational college), n (%)	51 (32%)	53 (33%)	10 (13%)	13 (24%)
Medium (college graduate or junior vocational college), n (%)	70 (43%)	73 (46%)	35 (46%)	23 (38%)
Low (no education or primary education), n (%)	41 (25%)	34 (21%)	31 (41%)	19 (35%)
Maternal smoking during pregnancy (>5 cigarettes/day), n (%)	35 (20%)	25 (15%)	14 (18%)	11 (20%)
Maternal alcohol during pregnancy, n (%)	19 (11%)	26 (16%)	7 (9%)	5 (9%)

HOME: Home Observation for Measurement of the Environment; OOS: Obstetric Optimality Score; LCPUFA: Long-chain polyunsaturated fatty acids

* significant difference between participants and non-participants. Chi-square: 11.70; p=0.003

significant difference between participants and non-participants. Chi-square: 7.14; p=0.028

The NEPSY (18) assesses neuropsychological development of children using tasks with which children are often confronted in daily life. Scaled scores of six different subtests were combined to form scores on three functional domains: attention/executive function (Tower), language (Speeded naming and Comprehension of instructions), and memory and learning (Narrative memory, Memory for faces and Memory for names). The NEPSY is standardized on a large sample of children and has excellent inter-rater reliability. Verbal memory was further evaluated with the word pair subtest of the Children's Memory Scale, which assesses immediate and delayed recall and recognition of word pairs, summarized in a total score. The Children's Memory Scale has a moderate to high correlation with other tests evaluating memory function (19). In addition, parents filled in a questionnaire on ADHD based on the criteria of the DSM-IV (20), which provided information on inattention.

All cognitive tests were scored according to the original non-Dutch norms, as Dutch norms are lacking. The raw scores achieved on the various tests were converted into scaled scores with a mean of 10 and a standard deviation of 3. Higher scores indicate better performance. The cognitive and neurological assessments were carried out by two psychologists (HKK and CdJ). The neurological assessment was supervised by MHA on the basis of the video-recording of the assessment. MHA was blind to results of the cognitive tests.

2.3. Statistical analyses

Power calculation of the original project had been based on scores on the Bayley Scales of Infant Development at 18 months. A post-hoc power analysis based on the outcomes of the NEPSY and TEA-Ch at school age indicated that at least28 children with fine manipulative disability and 27 children with dyscoordination were needed to detect a difference of half a SD (mean = 10 and SD = 3) with 80% power and alpha of 0.05.

Statistical analyses were carried out in three steps. The first step consisted of univariate analyses to determine differences in cognitive outcome between children with or without a specific type of dysfunction (Student t-test). In the second step, multivariate analysis was done to adjust for the influence of type of feeding during the first two months after birth and sex. Finally, multivariate analysis was performed to adjust for additional covariates. Factors known to influence cognition or neurodevelopment were entered into the regression analyses using backwards selection. The following variables were considered for the regression analyses: maternal verbal IQ, maternal and paternal education and profession, maternal smoking, alcohol and drug use during pregnancy, birth weight, Apgar scores at 1 and 3 min ,total OOS and the subcategories of the OOS summarizing the prenatal and social history. As performance in the domains memory and learning, verbal memory and language may be influenced by levels of attention, the inattention score of the ADHD-questionnaire

was included in multivariate analyses of memory and language. Two-tailed level of significance was set at 0.05. Statistical analyses were carried out with SPSS, version 16.0 (SPSS, Inc., Chicago IL).

3. Results

Dysfunction in the domain reflexes was the most frequently occurring type of MND (n = 159; 47%), followed by mild problems in coordination (n = 93; 27%). Fine manipulative disability was present in 86 children (25%), dysfunctional posture and muscle tone in 60 children (18%). Thirteen children (4%) showed choreiform dyskinesia, excessive associated movements occurred in 7 (2%) children and sensory deficits and cranial nerve dysfunction were present in one child. Three domains of dysfunction were associated with cognition: fine manipulative disability, coordination problems, and dysfunctional posture and muscle tone.

Fine manipulative disability was associated with lower scores on all cognitive functions (Table 2). Interestingly, all associations were statistically significant in the subgroup of boys (n = 55), but in girls (n = 31), only the associations with TEA-Ch sustained attention and the NEPSY domain learning and memory were statistically significant (Fig. 2). Multivariate analyses confirmed that children with fine manipulative disability had lower scores on all cognitive functions, except for the NEPSY domain attention/executive function. Details of the multivariate analyses of two cognitive outcomes are provided in Tables 3a and 3b. Results of the other cognitive outcomes were comparable (results not shown). The interactions with sex failed to reach statistical significance.

Children with coordination problems performed significantly worse than children without coordination problems on all cognitive tests, with the exception of the TEA-Ch selective attention task and the Children's Memory Scale word pairs task (Table 2). Contrary to the results in the domain of fine manipulative ability, the associations between coordination problems and cognition persisted mainly in girls and not in boys. In girls, coordination problems (n = 31) were related to lower scores on attentional switching, attention/executive functioning, memory and learning, language and on the Children's Memory Scale word pairs. Boys with coordination problems (n = 62) scored lower only on the memory and learning subtest (Fig. 3). Multivariate analyses confirmed univariate results for the entire group of children (examples in Tables 3a and 3b). Furthermore, the analyses demonstrated a significant interaction with sex for the attention/executive functioning subtest: girls with coordination problems performed significantly worse than those without this dys-

Table 2. *Fine manipulative disability and coordination problems and performance on neuropsychological tests.*

	Fine manipulative disability						Coordination problems					
	absent			Present			absent			Present		
	Mean	95% CI	n	Mean	95% CI	n	Mean	95% CI	n	Mean	95% CI	n
TEA-Ch Selective attention	7.74	7.4-8.1	255	6.82[b]	6.2-7.4	85	7.61	7.3-7.9	247	7.23	6.7-7.8	93
TEA-Ch Sustained attention	8.31	8.0-8.7	253	7.02[b]	6.4-7.7	83	8.23	7.9-8.6	244	7.78[a]	6.7-8.0	92
TEA-Ch Attentional switching	8.83	8.6-9.1	248	7.36[b]	6.7-8.0	79	8.72	8.5-9.0	241	7.78[b]	7.2-8.4	86
NEPSY Attention/ executive functioning	10.56	10.3-10.9	255	9.86[a]	9.3-10.5	86	10.59	10.3-10.9	248	9.84[a]	9.3-10.4	93
NEPSY Memory + learning	9.13	8.9-9.4	255	7.77[b]	7.3-8.2	84	9.05	8.8-9.3	247	8.12[b]	7.6-8.6	92
CMS Word pairs	8.52	8.1-8.9	250	7.22[b]	6.5-7.9	80	8.33	7.9-8.7	241	7.86	7.2-8.5	89
NEPSY Language	10.20	10.0-10.5	254	9.01[b]	8.5-9.5	86	10.09	9.8-10.3	248	9.40[a]	8.9-10.0	92

[a] t-test : $p<0.05$, [b] t-test : $p<0.01$

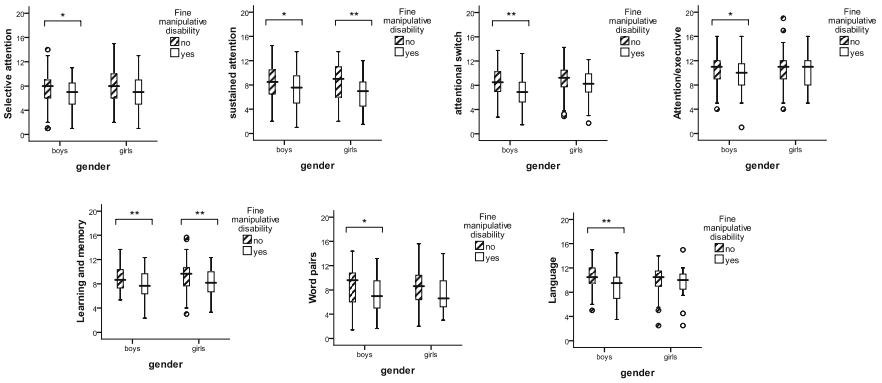


Figure 2. Cognitive outcome and fine manipulative disability in boys and girls.

function, whereas in boys no relation was found between coordination problems and attention/executive function (Table 4). For the other cognitive tasks, the interaction with sex could not be confirmed.

Dysfunction of posture and muscle tone was not associated with performance on cognitive tasks in the entire group of children. However in girls, dysfunctional posture and muscle tone was associated with lower scores on the NEPSY language scale (t-test; p = 0.006). This was confirmed by a significant interaction with sex after adjusting for type of feeding (Girls: B: -1.425; 95%CI: -2.47; -0.36; p = 0.009; boys: B:0.02; 95%CI: -0.72;0.77; p = 0.954). However, the interaction lost statistical significance after adjusting for additional covariates.

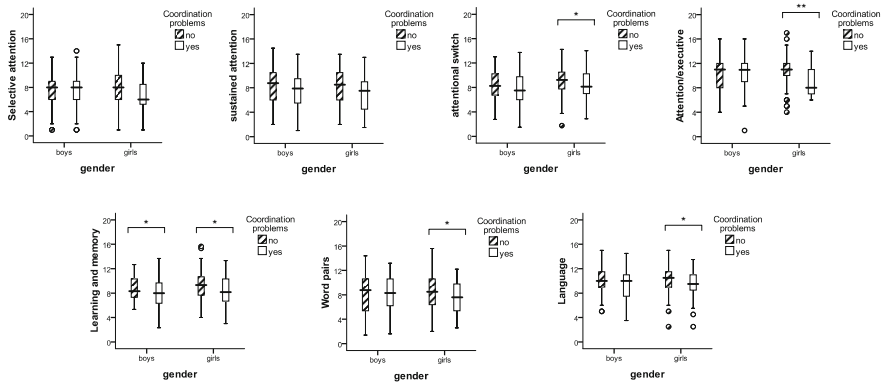


Fig. 3. Cognitive outcome and coordination problems in boys and girls.

Table 3a. *Multiple linear regression analyses for TEA-Ch attentional switching (n=327)*

	Fine manipulative disability					Coordination problems			
	Effect	**95% CI**	**p-value**	**R2**		**Effect**	**95% CI**	**p-value**	**R2**
Step 2: Type of feeding & sex				0.10	Step 2: Type of feeding & sex				0.07
Fine manipulative disability present vs absent	-1.26	-185– -0.67	<0.001		**Coordination problems present vs absent**	-0.74	-1.32– -0.16	0.013	
Type of feeding: LF vs CF (ref)	-0.18	-0.82– 0.46	0.585		Type of feeding: LF vs CF (ref)	-0.16	-0.81– 0.49	0.622	
BF vs CF	0.47	-0.12–1.05	0.115		BF vs CF	0.61	0.02–1.20	0.043	
BF vs LF	0.65	0.02–1.27	0.043		BF vs LF	0.77	0.14–1.40	0.017	
Female vs male	0.70	0.19–1.20	0.007		Female vs male	0.72	0.21–1.24	0.006	
	Effect	95% CI	p-value	R^2		Effect	95% CI	p-value	R^2
Step 3: additional covariates				0.15	Step 3: additional covariates				0.12
Fine manipulative disability present vs absent	-1.23	-1.80– -0.66	<0.001		**Coordination problems present vs absent**	-0.68	-1.25– -0.12	0.019	
Type of feeding: LF vs CF (ref)	-0.23	-0.86 – 0.39	0.461		Type of feeding: LF vs CF (ref)	-0.22	-0.85–0.41	0.496	
BF vs CF	0.20	-0.38 – 0.78	0.491		BF vs CF	0.34	-0.25–0.93	0.254	
BF vs LF	0.44	-0.18–1.05	0.174		BF vs LF	0.56	-0.06–1.18	0.078	
Paternal profession	1.17	0.65–1.70	<0.001		Paternal profession	1.17	0.63–1.71	<0.001	
Female vs male	0.73	0.24–1.21	0.004		Female vs male	0.76	0.26–1.26	0.003	

BF / LF / CF: Breastfed / LCPUFA supplemented / Control formula group; Paternal profession dichotomized as: middle/high vs low

Paternal profession: n= 325; (statistically significant difference in paternal profession at 18 months between cases with available information on paternal profession at 9 years and missing data: χ^2= 5.387; p=0.020. If data on paternal profession was missing at 9 years, this was substituted by paternal profession at 18 months)

Table 3b. *Multiple linear regression analyses for NEPSY memory and learning*

	Fine manipulative disability					Coordination problems			
	Effect	**95% CI**	**p-value**	**R2**		**Effect**	**95% CI**	**p-value**	**R2**
Step 2: Type of feeding & sex (n=339)				0.13	Step 2: Type of feeding & sex (n=339)				
Fine manipulative disability present vs absent	-1.12	-1.65– -0.60	<0.001		**Coordination problems present vs absent**	-0.78	-1.30– -0.26	0.003	
Type of feeding: LF vs CF (ref)	0.08	-0.49–0.66	0.777		Type of feeding: LF vs CF (ref)	0.09	-0.49–0.67	0.762	
BF vs CF	1.12	0.60–1.64	<0.001		BF vs CF	1.24	0.71–1.76	<0.001	
BF vs LF	1.04	0.47–1.61	<0.001		BF vs LF	1.15	0.57–1.72	<0.001	
Female vs male	0.40	-0.05–0.85	0.083		Female vs male	0.40	-0.06–0.86	0.087	
	Effect	95% CI	p-value	R^2		Effect	95% CI	p-value	R^2
Step 3: additional covariates (n=299)				0.28	Step 3: additional covariates (n=299)				0.26
Fine manipulative disability present vs absent	-0.97	-1.48– -0.47	<0.001		**Coordination problems present vs absent**	-0.60	-1.10– -0.10	0.019	
Type of feeding: LF vs CF (ref)	0.16	-0.40– 0.72	0.577		Type of feeding: LF vs CF (ref)	0.13	-0.43–0.70	0.643	
BF vs CF	0.54	-0.01–1.09	0.052		BF vs CF	0.60	0.04–1.16	0.034	
BF vs LF	0.39	-0.20–0.96	0.192		BF vs LF	0.47	-0.12–1.05	0.119	
Inattention	-0.07	-0.11– -0.03	<0.001		Inattention	-0.07	-0.11– -0.04	<0.001	
Paternal education	1.13	0.59–1.67	<0.001		Paternal education	1.14	0.60–1.69	<0.001	
Maternal VIQ	0.03	0.01–0.04	0.002		Maternal VIQ	0.03	0.01–0.05	0.001	
Maternal smoking during pregnancy	-0.66	-1.26– -0.06	0.032		Maternal smoking during pregnancy	-0.67	-1.28– -0.06	0.033	

BF / LF / CF: Breastfed / LCPUFA supplemented / Control formula group

Maternal smoking during pregnancy dichotomized as 0-5 cigarettes/day vs >5 cigarettes/day. Paternal profession dichotomized as: middle/high vs low

Maternal Verbal IQ: n=312; (no statistically significant difference in maternal education between cases with available information on maternal VIQ and missing data for maternal VIQ: χ^2=1.313; p=0.567).

Paternal education: n= 325; (no statistically significant difference in paternal education at 18 months between cases with available information on paternal education at 9 years and missing data: χ^2= 3.456; p=0.063. If data on paternal education was missing at 9 years, this was substituted by paternal education at 18 months)

Table 4. *Multiple linear regression analyses for coordination problems and NEPSY attention/executive functioning.*

	Effect	95% CI	p-value
Dysfunctional coordination within sex			
Boys: dysfunction vs no dysfunction	-0.100	-0.873;0.674	0.800
Girls: dysfunction vs no dysfunction	-1.738	-2.720;-0.757	0.001
sex within dysfunctional domain coordination			
Non-dysfunctional coordination: girls vs boys	0.517	-0.110;1.144	0.106
Dysfunctional coordination: girls vs boys	-1.122	-2.205;-0.039	0.042
Type of Feeding			
LF vs CF (reference)	-1.052	-1.734;-0.370	0.003
BF vs CF	0.008	-0.615;0.632	0.979
BF vs LF	1.060	0.384;1.736	0.002

n=341 R^2= 0.07

4. Discussion

The present study is the first study to demonstrate associations between two specific types of MND and worse performance in specific cognitive domains at school age in low risk children born at term: fine manipulative disability and coordination problems were associated with worse performance in the domains of attention, learning and memory and language. These results are in concordance with the finding that children with DCD show impairments on tasks in working memory, language and executive functions (21), since children with poor motor abilities often show problems in the domains of fine manipulative ability and coordination (10). Of the two types of MND, fine manipulative disability was most consistently associated with cognitive impairments, which is in line with previous studies (4,8). Other types of MND were not associated with cognitive impairments. Although this may have been the result of a lack of power for these domains (9), it is in concordance with Soorani-Lunsing et al. (8), who reported associations between learning problems and fine manipulative disability and coordination problems.

It has been suggested that MND, and in particular fine manipulative disability and coordination problems, is a manifestation of cortico- striato-thalamo-cortical and cerebello-thalamo-cortical dysfunction caused by prenatal and perinatal adversities, such as preterm birth (3). The former pathway has been associated with movement control and sequential finger movement tasks (22) and the latter with motor learning and timing of movements (1). These brain circuitries may be especially vulnerable for

subtle adversities as the maturation of the frontal-striatal systems and of the cerebellum lasts throughout childhood until adolescence (23) which is in parallel with the development of higher-order cognitive function. The cognitive functions associated with the cortico-striato-thalamo-cortical pathways include spatial working memory, planning and attentional switching and tasks involving planning, language and set-shifting are associated with the cerebello- thalamo-cortical pathways (24). Therefore, it is likely that dysfunction of these pathways results not only in motor problems, but also in cognitive deficits in the domains of attention, memory and language.

Our study was carried out in children born at term without a specific risk for developmental disorders. We expect that the associations found in the present study also will be present – and perhaps stronger – in children neonatally considered as high risk, as these high risk infants have a higher prevalence of lesions of complex neural circuitries, MND and cognitive dysfunction (25–28).It is interesting that we found the associations between specific types of MND and cognition in children without perinatal or neonatal risk. This may suggest that subtle events occurring during early phases of ontogeny play a role in the origin of specific forms of MND and reduced cognitive function. The early origin is for example illustrated by the association of minor physical anomalies and fine manipulative dysfunction (27). Another prenatal source of subtle alterations of neurocognitive circuitries is maternal stress during pregnancy. Being exposed to prenatal stress increases the risk of MND and non-optimal cognitive performance (29,30). Whether or not these factors played a role in the current study group is unknown. Interestingly, in our study prenatal, perinatal and neonatal factors played a minimal confounding role, with maternal smoking during pregnancy being the only exception to the rule. A final explanation may be that subtle long-lasting environmental factors influence the development of complex cortico-subcortical circuitries, due to the vulnerability of these circuitries as a consequence of their protracted maturation. This idea is supported by the fact that paternal education and maternal IQ were major confounders in the associations between MND and cognition. Several explanations may be offered for the contribution of these factors. Firstly, parental education may influence child achievement directly via genetic factors. Furthermore, parental education may be associated with behaviors that influence child achievement. This effect may already operate in utero, for example maternal smoking during pregnancy has been related to lower levels of education as well as lower cognitive outcome in children (31). Finally, higher levels of parental cognition may lead to higher cognitive performance indirectly by creating a stimulating environment offering more learning experiences (32,33).

The results further indicated some sex differences in the associations between MND and cognitive functions. In the univariate analyses, it seemed that in girls in particular coordination problems are associated with cognitive problems, whereas in boys fine manipulative disability is associated with worse cognitive performance.

This is in line with previous results, demonstrating that boys with fine manipulative disability had a lower performance IQ, whereas in girls fine manipulative disability was not associated with performance IQ (9). Interestingly, imaging studies also suggest small sex differences in brain development. Boys tend to have an overall larger brain volume than girls and specifically relatively larger amygdala, hippocampus and cerebellum, whereas girls have relatively larger caudate nucleus and globus pallidus than boys (34). In addition, sex specific developmental trajectories of cortical and subcortical brain areas, including the frontal lobe and caudate nucleus, have been described. Girls tend to reach peak sizes earlier than boys (12) and appear to employ a more mature pattern of the front-striatal pathways compared with boys (35). It could be surmised that the sex differences in brain maturation result in differences in the sequelae of similar adverse events. These adverse events may also be associated with MND. Support for this theory comes from animal research, which has demonstrated that prenatal stress differentially affects the male and female rat brain (36). On the basis of the results we suggest the following hypothesis: in girls mild dysfunction of the cerebello-thalamo-cortical pathways is associated with cognitive impairment, whereas in boys mild dysfunction of cortico-striato- thalamo-cortical pathways is associated with increased vulnerability for cognitive problems. The precise mechanisms through which sex differences in brain maturation may translate to differences in the associations between neurological development and cognition are not known and follow-up on possible sex differences in neurocognitive associations should be done to confirm these suggestions.

4.1. Strengths and limitations

Strengths of the current study include the use of a standardized, age-specific detailed neurological examination, the assessment of a broad range of cognitive domains using well validated test measures, and – according to the post-hoc power analysis – adequate power. The examinations were applied in a relatively large group of children born at term in which information of many background factors, including maternal IQ was available. Even though the exclusion of children with perinatal risk may imply that the results cannot be extrapolated to groups of high risk infants, the exclusion of high risk children may also be regarded as a strength, as it offered the opportunity to explore neurocognitive associations in a population without well-known perinatal adversities.

It may be considered as a limitation of the study that it was based on children participating in a randomized controlled trial on the effects of supplementation of formula with LCPUFA during two months after birth. One of the results of the study design was that only 37% of the children were breastfed after birth, compared with 78% in a typical Dutch population (37). Breastfeeding has been associated with a better neurological condition (5,38) and with less fine manipulative disability (13),

which may have resulted in the high prevalence of MND found in this study compared to previous results (4). However, whether this effect is a result of the type of feeding or due to factors associated with breastfeeding, such as maternal education or environmental factors, is unknown. In order to adjust for a potential effect of type of feeding on neurological development, multivariate analyses were performed.

5. Conclusion

Fine manipulative disability and coordination problems in children born at term were associated with cognitive impairment in the domains of attention, learning and memory and language. These findings may be relevant in particular for children presenting with motor problems, i.e. DCD, as these children often show MND (10). Our data suggest that it is advisable to assess the profile of cognitive functions in children with DCD presenting during the neurological examination with fine manipulative disability or coordination problems. This offers the possibility of early detection of cognitive dysfunction, thereby creating opportunities for timely intervention.

6. Key points

- In children with developmental disorders, motor problems are often associated with cognitive difficulties. The associations between specific cognitive deficits underlying learning problems and minor neurological dysfunction (MND) are unknown.
- The study indicates that in particular fine manipulative disability and coordination problems are associated with impaired attention, memory and learning and language.
- Previous and present data suggest the existence of sex-specific neurological vulnerability for cognitive impairment: in girls mild dysfunction of the cerebello-thalamo-cortical pathways may be associated with cognitive impairment, whereas in boys mild dysfunction of cortico-striato-thalamo-cortical pathways may be associated with increased vulnerability for cognitive problems.
- The findings may be relevant for children with developmental coordination disorder.

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Chapter 5

Minor neurological dysfunction and behaviour in 9-year-old children born at term: evidence for sex dimorphism

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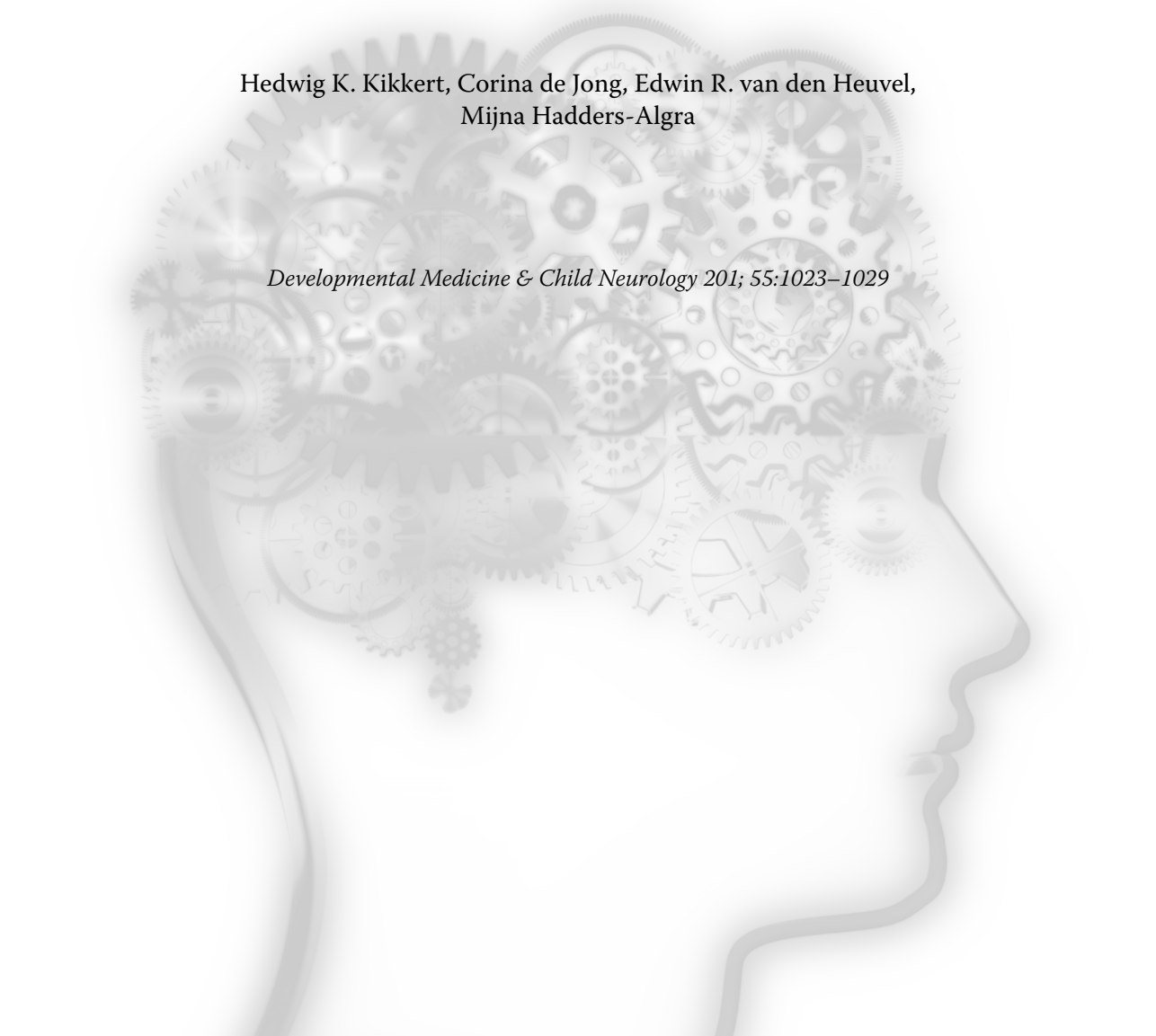

Abstract

Aim: The aim of the study was to assess associations between minor neurological dysfunction (MND) and behaviour, with specific attention to sex differences.

Method: This was an observational cohort study in which 341 9-year-old children (177 male, 164 female) without perinatal risk were neurologically assessed, with attention to severity and type of MND. Eight domains of dysfunction were distinguished, including posture and muscle tone, fine manipulative ability and coordination. Severity of MND was based on the number of dysfunctional domains. Behaviour was assessed by parents and teachers using the Child Behavior Checklist and Teacher's Report Form (TRF); outcomes were internalizing and externalizing behaviour and total score of behavioural problems.

Results: Females with complex MND or dysfunctional posture and muscle tone had increased risk for externalizing behavioural problems (OR 4.52, 95% CI 1.01–20.2, and OR 4.05, 95% CI 1.06–15.5, respectively). In males, these associations were absent. However, males with simple MND had an increased risk for behavioural problems indicated by the total TRF-score (OR 7.52, 95% CI 1.36–41.4).

Interpretation: In children without perinatal risk, associations between MND and behaviour are sex-specific. In females, dysfunction of complex neural networks, including the cerebellum, is associated with externalizing behaviour. In males, neurobehavioural relationships are limited, suggesting a larger role of psychosocial factors in the genesis of behavioural problems.

What this paper adds:
- In 9-year-olds without perinatal risk neurobehavioural associations are sex-specific.
- In girls, complex MND is associated with externalizing behavioural problems.
- This association is brought about particularly by dysfunctional posture.
- In boys, simple MND is associated with unspecific behavioural problems

1. Introduction

In recent decades, attention to developmental coordination disorder and its associated impairments, such as behavioural problems, has increased (1–3). However, the underlying substrate for the association between motor impairments and behavioural problems is still unknown.

The finding that motor problems are related to impairments in other domains, such as behaviour, has led to growing interest in minor neurological dysfunction (MND) and its correlates. MND has been defined as the occurrence of neurological dysfunction in the absence of evident neurological pathology, such as cerebral palsy. MND can be expressed in terms of type of MND, such as dysfunctional posture and muscle tone or dyscoordination, and severity of MND, which is expressed as simple (s-MND) or complex MND (c-MND). At school age the distinction between simple and complex MND is based on thenumber of dysfunctional domains. s-MND denotes the presence of one or two dysfunctional domains, whereas c-MND implies the presence of at least three dysfunctional domains. s-MND reflects a typical, but non-optimal form of brain function (minor neurological difference). It occurs in about 20% of children and is associated with prenatal and perinatal stress, such as intrauterine growth retardation and preterm birth without additional complications, a slightly lower IQ and a mild increase in behavioural problems (4,5). Conversely, c-MND, which occurs in about 5% of children, may be regarded as the clinically relevant form of MND on account of its strong relationships with perinatal adversities and learning and behavioural problems (4).

Specific types of MND, such as dysfunctional posture and muscle tone, fine manipulative disability, dyscoordination and dyskinesia have been associated with behaviour in the form of attention problems, fearfulness, clumsiness, social problems and internalizing and externalizing behaviour (6–8). However, the relation between MND and specific behavioural problems has not yet been assessed in a typical population without perinatal risk using well-normed assessments.

Therefore, the aim of the study was to assess the association between severity and type of MND and behaviour in a healthy population of 9-year-old children born at term using standardized, normed tests. The advantage of a healthy group, that is children without specific perinatal risk, is that potential neurobehavioural associations are not induced by perinatal adversities.

We also were interested in possible sex differences in neurobehavioural associations, as the prevalence of developmental disorders, such as ADHD differs for males and females (9) and neuroimaging studies suggest sex differences in human brain development (10–12). Previously, we established interactions between sex and MND for IQ and cognition. The studies demonstrated that in males fine manipulative disability was associated with lower IQ (5), and in females dyscoordination was associated

with cognitive impairments (13). These results have led to the hypothesis that in males specifically fine manipulative disability will be associated with behavioural problems, whereas in females dyscoordination is expected to be related to behavioural problems.

2. Method

2.1 Participants

Three hundred and forty-one participants (177 males, 164 females) of the Groningen LCPUFA project were assessed at 9 years of age (range 8y 10mo–9y 7mo; 72% of original cohort). The project was originally designed to examine effects of supplementation of formula with long-chain polyunsaturated fatty acids (LCPUFA) during the first two postnatal months. Infants were randomized to receive either formula with LCPUFA (Nutrilon Premium with 0.45% (by wt) AA and 0.30% (by wt) DHA) or control formula without LCPUFA. A third group of infants was breastfed (for details, see de Jong et al.)(14). At enrolment, extensive information on social, prenatal and perinatal conditions was collected, which allowed us to form an obstetric optimality score (OOS) (15). In addition, social background was evaluated using the Home Observation for Measurement of the Environment (HOME) (16).

At 9 years of age, information on MND, behaviour and data on current social situation was collected. Characteristics of participating and non-participating children are presented in Table 1.

2.2 Procedures

Neurological condition was evaluated by a standardized and age-specific technique designed to assess MND, taking developmental changes into account (17,18). The examination assesses eight domains of dysfunction: fine manipulative ability, coordination, choreiform dyskinesia, posture and muscle tone, reflexes, associated movements, sensory deficits and cranial nerve function. A domain is considered dysfunctional in the presence of multiple items with a dysfunctional performance (19). Based on the number of dysfunctional domains, a distinction can be made between s-MND and c-MND: the presence of three or more dysfunctional domains signifies the presence of c-MND and the presence of one or two dysfunctional domains implies s-MND. The child is classified as neurologically normal in the absence of a dysfunctional domain or in case of the isolated presence of dysfunctional reflexes. Inter-rater, intra-rater and test–retest reliability for neurological classification varies from good to excellent (19).

Table 1. *Demographic characteristics of participants (n=341), non-participants (n=133) and their parents.*

	Participants		**Non-participants**	
	Boys (n=177)	**Girls (n=164)**	**Boys (n=78)**	**Girls (n=55)**
Birth weight (g), mean (SD)	3570 (497)	3515 (427)	3573 (405)	3450 (463)
Apgar score at 3 minutes, median (range)	10 (5-10)	10 (7-10)	10 (7-10)	10 (9-10)
Missing, n (%)	15 (8%)	15 (9%)	16 (21%)	16 (29%)
HOME, median (range)	43 (33-45)	44 (32-45)	43 (36-45)	43 (38-45)
Missing, n (%)	5 (3%)	2 (1%)	43 (56%)	38 (69%)
OOS, median (range)	59 (43-69)	59 (43-69)	58 (50-68)	61 (47-67)
Firstborn	41%	51%	40%	38%
Type of feeding[*]				
Breastfed	36%	38%	19%	31%
LCPUFA	24%	30%	46%	35%
Standard formula	40%	32%	35%	35%
Maternal education[^#]				
High	27%	27%	13%	15%
Medium	59%	59%	58%	46%
Low	14%	15%	29%	40%
Missing, n (%)	7 (4%)	6 (4%)	0 (0%)	0 (0%)
Paternal education[$]				
High	31%	33%	13%	24%
Medium	43%	46%	46%	42%
Low	25%	21%	41%	35%
Missing, n (%)	15 (8%)	4 (2%)	2 (3%)	0 (0%)
Maternal smoking	20%	15%	18%	20%
Maternal alcohol	16%	18%	9%	9%

[*] Significant difference between male participants and non-participants ($\chi^2 = 14.3$, df=2, p<0.001)

[^] Significant difference between male participants and non-participants ($\chi^2 = 12.2$, df=2, p=0.002)

[#] Significant difference between female participants and non-participants ($\chi^2 = 16.3$, df=2, p<0.001)

[$] Significant difference between male participants and non-participants ($\chi^2 = 11.0$, df=2, p=0.004)

HOME, Home Observation for Measurement of the Environment; OOS, obstetric optimality score: LCPUFA, long-chain polyunsaturated fatty acids.

Behaviour was assessed using the Child Behavior Checklist (CBCL) (20) and the Teacher's Report Form (TRF) (20), resulting in an assessment of the child's behaviour at home and at school respectively. We focused on three outcomes of both CBCL and TRF: externalizing and internalizing behaviour and total problem scores. The scores were dichotomized into healthy or borderline/clinical behaviour in accordance with the manual (further denoted as 'behavioural problem'). Missing items were processed according to the manual. The CBCL and TRF have good reliability and validity (20,21). IQ was tested with the Wechsler Abbreviated Scale of Intelligence (WASI) (5).

2.3 Statistical analyses

Differences between demographic characteristics of the participants and non-participants were tested for males and females separately with the Wilcoxon rank sum test and the χ^2 test for numerical and categorical variables respectively.

The difference between males and females in severity of MND was tested with Pearson's χ^2 statistics (at significance α=0.01). Sex differences in the binary outcomes of the types of MND were simultaneously modelled with a generalized linear mixed effects model using the GLIMMIX procedure of SAS, its default estimation method for pseudo-likelihood and overdispersion. A random intercept for males and females separately was implemented and fixed effects were used for the types of MND, sex and their interaction (between type of MND and sex). Specific contrast statements were applied to test for differences between males and females on each individual type of MND. The same model was used to analyse the behavioural outcomes simultaneously and to analyse the internalizing and externalizing outcomes simultaneously. CBCL and TRF data were analysed separately.

In the analyses on the associations between MND and behaviour, the types of MND were limited to fine manipulative disability, dyscoordination and dysfunctional posture and muscle tone, as the prevalences of choreiform dyskinesia (n=13), excessive associated movements (n =7), cranial nerve dysfunction (n =1) and sensory deficits (n =1) precluded the evaluation of relationships between these domains and behavioural problems. Results for the domain reflexes were omitted, as isolated presence of dysfunction in this domain does not have clinical significance.

Logistic regression analysis was applied to investigate the effect of severity of MND and each behavioural problem domain separately, and whether this was the same for both sexes. This analysis was also performed for each type of MND. OR with 95% CI of the effect of severity and type of MND were provided per sex. In the logistic regression analyses, the effects were corrected for birthweight, gestational age, postnatal type of feeding, maternal and paternal education, maternal smoking and alcohol use during pregnancy and the HOME. Postnatal type of feeding was

included, as the original study was designed to study the effect of postnatal nutrition and it is known that breastfeeding is associated with a better neurological condition at 9 years than formula feeding (14,22). The study design was approved by the ethics committee of the University Medical Center Groningen. All parents provided written informed consent.

3. Results

3.1 Prevalence of behavioural problems and MND

Raw data on MND and behavioural problems are summarized in Tables 2 and 3. The CBCL and TRF data indicated that males more often showed externalizing behavioural problems than females. Males also more often showed problem behaviour on the total score of the CBCL than females (Table 2). Also, with respect to neurological condition, males more often showed impairments than females. They more often had MND, in particular fine manipulative disability, dyscoordination and dysfunctional posture and muscle tone (Table 3). Neither the presence of externalizing and internalizing behavioural problems nor problem behaviour on the total scores on CBCL and TRF was associated with IQ scores in males or females.

3.2 MND and behavioural problems in females

We present only data corrected for confounders. The CBCL data indicated that females with c-MND more often had externalizing behavioural problems than neurologically normal females (adjusted OR 4.52, 95% CI 1.01–20.2; Table 4). The risk of females with s-MND for externalizing behavioural problems was similar to that of neurologically normal females. The association between MND and externalizing behaviour on the CBCL was brought about in particular by the association with dysfunctional posture and muscle tone (OR 4.05, 95% CI 1.06–15.5; Table 5). Dyscoordination and fine manipulative disability were not associated with an increased risk for behavioural problems. In females, severity and type of MND were not associated with problem behaviour on internalizing behaviour and total problem scores on the CBCL, nor with behavioural problems on the TRF. MND and behavioural problems in males Again we present only data adjusted for confounders. In males, s-MND was associated with behavioural problems on the total problem score of the TRF (adjusted OR 7.52, 95% CI 1.36–41.4; Table 4). Neither the presence of c-MND nor that of specific types of MND were associated with problem behaviour, either at home or at school (Tables 4 and 5).

Table 2. *Prevalence of behavioural problems in boys and girls.*

	CBCL behaviour problems			TRF behaviour problems		
	Boys	**Girls**	**P-value**	**Boys**	**Girls**	**P-value**
Internalizing behaviour	48 (27%)	29 (18%)	0.061	18 (14%)	24 (20%)	0.114
Externalizing behaviour	52 (29%)	14 (9%)	<0.001	25 (20%)	13 (11%)	0.002
Total problem score	48 (27%)	25 (15%)	0.008	24 (19%)	15 (13%)	0.168

Table 3. *Prevalence of various forms of minor neurological dysfunction in boys and girls.*

	Boys (n=177)	**Girls (n=164)**	**P-value**
Severity of MND			
Normal	70 (40%)	100 (61%)	
Simple	76 (43%)	50 (30%)	<0.001
Complex	31 (18%)	14 (9%)	
Dysfunctional reflexes	84 (47%)	75 (46%)	0.740
Dyscoordination	62 (35%)	31 (19%)	<0.001
Fine manipulative disability*	55 (31%)	31 (19%)	0.005
Dysfunctional posture & muscle tone	42 (24%)	18 (11%)	<0.001
Choreiform dyskinesia	10 (6%)	3 (2%)	0.044
Excessive associated movements	5 (3%)	2 (1%)	0.241
Sensory deficits	0 (0%)	1 (1%)	NA
Cranial nerve dysfunction	1 (1%)	0 (0%)	NA

* The domain fine manipulative disability was the only neurological domain affected by infant nutrition. Fine manipulative disability occurred in 17% of breastfeed children and in 30% and 31% of the children fed with formula with and without LCPUFA supplementation, respectively. Breastfed versus formula groups: p=0.002, for details see [14]. NA, not available.

Table 4. *Adjusted odds ratios of simple and complex MND for behavioural problems in boys and girls.*

	Boys		Girls	
	Simple MND	**Complex MND**	**Simple MND**	**Complex MND**
CBCL Total Score	2.12 [0.90 ; 5.00]	1.07 [0.34 ; 3.38]	1.21 [0.43 ; 3.45]	3.57 [0.92 ; 13.8]
CBCL Internalizing	1.93 [0.83 ; 4.52]	2.06 [0.70 ; 6.12]	1.51 [0.60 ; 3.83]	1.09 [0.20 ; 5.82]
CBCL Externalizing*	1.64 [0.74 ; 3.64]	0.57 [0.18 ; 1.80]	0.83 [0.19 ; 3.64]	**4.52 [1.01 ; 20.2]**
TRF Total Score	**7.52 [1.36 ; 41.4]**	1.70 [0.17 ; 16.5]	4.14 [0.94 ; 18.3]	0.87 [0.05 ; 15.1]
TRF Internalizing	1.00 [0.28 ; 3.53]	0.92 [0.17 ; 5.04]	2.25 [0.78 ; 6.52]	0.39 [0.03 ; 4.64]
TRF Externalizing	2.02 [0.55 ; 7.45]	0.49 [0.06 ; 4.20]	4.02 [0.93 ; 17.3]	0.99 [0.07 ; 14.8]

Children with MND were compared to neurologically normal children. Adjustment was carried out for birth weight, gestational age, type of feeding after birth, maternal and paternal education, maternal smoking and alcohol use during pregnancy and the HOME; missing cases: n=24

Bold: statistically significant associations

*Interaction effect between gender and severity of MND was significant at 0.05.

MND, minor neurological dysfunction; CBCL, Child Behavior Checklist; TRF, Teacher's Report Form.

Table 5. *Adjusted odds ratios of specific types of MND for behavioral problems in boys and girls.*

		Dyscoordination	**Fine manipulative disability**	**Dysfunctional posture & muscle tone**
CBCL Total Score	Boys	1.01 [0.47 ; 2.21]	1.38 [0.63 ; 3.01]	1.07 [0.45 ; 2.56]
	Girls	2.04 [0.73 ; 5.70]	1.05 [0.34 ; 3.25]	3.16 [0.99 ; 10.1]
CBCL Internalizing	Boys	0.97 [0.44 ; 2.10]	1.29 [0.59 ; 2.82]	1.49 [0.63 ; 3.52]
	Girls	1.51 [0.53 ; 4.29]	0.88 [0.28 ; 2.73]	1.10 [0.27 ; 4.43]
CBCL Externalizing*	Boys	0.72 [0.34 ; 1.54]	0.75 [0.34 ; 1.62]	0.73 [0.31 ; 1.74]
	Girls	1.60 [0.44 ; 5.86]	0.67 [0.14 ; 3.25]	**4.05 [1.06 ; 15.5]**
TRF Total Score	Boys	0.62 [0.19 ; 2.04]	2.21 [0.71 ; 6.90]	1.43 [0.41 ; 5.02]
	Girls	0.96 [0.21 ; 4.32]	1.31 [0.27 ; 6.39]	1.81 [0.37 ; 8.88]
TRF Internalizing	Boys	0.29 [0.07 ; 1.20]	2.20 [0.68 ; 7.17]	0.44 [0.08 ; 2.37]
	Girls	0.56 [0.14 ; 2.32]	1.06 [0.27 ; 4.12]	1.97 [0.48 ; 8.07]
TRF Externalizing	Boys	0.66 [0.20 ; 2.18]	1.02 [0.32 ; 3.28]	0.76 [0.18 ; 3.12]
	Girls	1.20 [0.26 ; 5.56]	0.80 [0.13 ; 4.77]	2.69 [0.51 ; 14.3]

Adjustment was carried for birth weight, gestational age, type of feeding after birth, maternal and paternal education, maternal smoking and alcohol use during pregnancy and the HOME; missing cases: n=24

Bold: statistically significant associations

*Interaction effect between gender and dysfunctional posture & muscle tone was significant at 0.05.

MND, minor neurological dysfunction; CBCL, Child Behavior Checklist; TRF, Teacher's Report Form.

4. Discussion

Our data indicated that in females born at term, c-MND and dysfunctional posture and muscle tone were associated with an increased risk of externalizing behavioural problems reported by parents. In males, presence of s-MND was associated with increased risk for unspecific behavioural problems at school; specific domains of dysfunction were not related to behavioural problems in males.

The association of MND with behavioural problems is in concordance with previous studies (6,7). However, previously attention problems and internalizing behaviour were also related to MND, and specifically to fine manipulative disability, dyscoordination, dysfunctional posture and muscle tone and choreiform dyskinesia (6,7). The association between dysfunctional posture and muscle tone and behaviour was replicated in this study; however, associations with other types of MND did not reach statistical significance. Firstly, this may be explained by the low absolute number of children with MND and behavioural problems. For example, only 14 females and 31 males were classified with c-MND; of these children, only five females and eight males also had behavioural problems on the total CBCL score. Consequently, the study may have lacked power to detect associations between MND and behaviour. Secondly, and more importantly, previous studies included children with perinatal risk. It may be that neurobehavioural associations depend on the degree of perinatal risk, as the prevalence of lesions of complex neural circuitries, MND and behavioural problems is higher in children with perinatal risk factors (23–25). The healthy nature of our population may also have caused the absence of the clinically known association between behavioural problems and IQ (26).

The typical nature of our population, that is the absence of perinatal risk and its associated neurological pathology, may have allowed us to uncover sex-specific neurobehavioural associations which have not been reported previously. Sex-specific associations between behaviour and neural substrate had been reported by neuroimaging studies. For example, aggressive behaviour has been associated with smaller volume of the anterior cingulate cortex only in males (27), and hippocampal volume was associated with aggressive behaviour only in females (28). We demonstrated currently that, in females, c-MND and dysfunctional posture and muscle tone were associated with externalizing behaviour; in males, these associations were absent. It has been suggested that the neural substrate of c-MND is dysfunction of complex cortico-striato-thalamo-cortical and cerebello-thalamo-cortical pathways (4), as motor functions associated with these pathways are movement control and sequential finger movement tasks (29), and motor learning and timing of movements respectively (30). Posture and muscle tone regulation involve major parts of the central nervous system, in which subcortical structures such as the cerebellum play a large role (18). In males, s-MND was associated with behavioural problems

on the total problem score. S-MND is associated with stress during early life, which may induce alterations in the monoaminergic systems of the brain which have diffuse projections throughout the nervous system (4). As a result of the role of these brain structures in motor function and behaviour, it is conceivable that mild dysfunction in these systems manifests itself in unspecific forms of MND and unspecific behavioural problems.

In line with the literature (4,9), we found that the prevalence of MND and behavioural problems was higher in males, implying that males are more vulnerable to developmental disorders. This may be explained by sex differences in brain development. In general, the brains of females mature faster than those of males (10). As a result, the period during which the developing male brain may be exposed to adverse effects of environmental events, including stress, is longer than that of female brains. Consequently, the male brain is more susceptible to the development of behavioural problems or MND than the female brain. Yet, in females, MND, in particular dysfunctional postural and muscle tone, co-occurs with externalizing behavioural problems. This finding is in line with recent MRI studies on sex differences in typical brain development, which demonstrated that males have a larger overall brain volume, cerebellum and amygdala than females (11,12), and that females have a disproportionately larger caudate nucleus, globus pallidus and hippocampus (11,12). Thus, it could be hypothesized that (1) males are more vulnerable to dysfunction of the basal ganglia and hippocampus, which was supported previously by the association between fine manipulative disability and lower IQ,5 and (2) females are more vulnerable to dysfunction of the cerebellum and amygdala, which would explain the associations between mild dysfunction of posture and muscle tone and behavioural problems reported in this study. Furthermore, it is possible that sex differences in typical brain development result in sex differences in response to adverse events. Evidence for this hypothesis may be found in animal research, demonstrating sex differences in response to stress (31–33).

Next to biological explanations for the associations between MND and behaviour, psychosocial explanations deserve attention. Children with motor difficulties often have lower self-esteem than their peers (34), which is associated with internalizing and externalizing behavioural problems (35). In particular, the association between s-MND and behavioural problems indicated by the total TRF scores in males suggests an effect of psychosocial factors. S-MND is regarded as a normal, albeit non-optimal, form of brain function, reflecting an unspecific mildly increased vulnerability for behavioural problems (4). This vulnerability differs from the more specific vulnerability for behavioural problems in females. In females, c-MND, with its stronger roots in perinatal adversities and a specific neurological domain (dysfunctional posture and muscle tone regulation), was associated with a specific type of behaviour (externalizing behaviour). This also suggests that in females presenting

with motor problems (who often have MND), screening for behavioural problems should be considered. Early detection of behavioural problems and risk for psychopathology allows for intervention and prevention of deterioration (36,37).

Interestingly, associations between MND and parent and teacher reports of behaviour differed, which is in line with the previously reported low agreement between parent and teacher ratings of behaviour (38). These discrepancies may be explained by differences in the settings in which behaviour is evaluated.

4.1 Strengths and limitations

Participants were recruited for a randomized controlled trial on the effects of supplementation of formula with LCPUFA during the 2 months after birth. Consequently, this influenced the composition of the study group, which may be considered a limitation as results therefore cannot be generalized to the whole population of Dutch 9-year old children. First, the percentage of children breastfed after birth was considerably lower than that among the typical Dutch population (37% vs 78%) (39). This may have resulted in a higher prevalence of MND in this study than previously reported (4), as it has been demonstrated that breastfed children have better neurological condition and less fine manipulative disability than formula-fed children (14,22,40). As mentioned before, our study may have lacked the power to demonstrate certain neurobehavioural associations. Secondly, during follow-up selective attrition occurred: at 9 years of age, children of highly educated parents were more likely to participate. This may have influenced results, as parental education may influence motor development (41). In order to minimize the effects of parental education and to adjust for a potential effect of type of feeding and environmental factors on neurobehavioural associations, multivariate analyses were performed. Lastly, children with perinatal risk were excluded from the study, meaning that the results cannot be extrapolated to high-risk infants. However, the trial offered the opportunity to study neurobehavioural associations in children without perinatal or neonatal risk. Strengths of the study include availability of information on many background and environmental factors, such as parental profession and the HOME. Additional strengths include the use of an age-specific detailed neurological examination and standardized assessments of behaviour, both at home and school.

5. Conclusion

Our study indicated that in children without specific perinatal risk, associations between MND and behaviour were sex specific. In females, neurobiologically specific associations were found: c-MND and dysfunctional posture and muscle tone were

associated with an increased risk of externalizing behavioural problems; these associations were absent in males. In contrast, in males a neurobiologically unspecific association was present: s-MND, that is the presence of a typical, but non-optimal neurological condition, was associated with an increase in unspecified behavioural problems. This suggests that in the genesis of behavioural problems, psychosocial factors play a larger role in males than in females. Our findings correspond to the increasing evidence for sex differences in brain development shown by neuroimaging studies. Future studies, including neuroimaging and neurological assessment in children with and without perinatal risk, may shed further light on sex-specific neurobehavioural relationships.

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Part III

General discussion and summary

Chapter 6

General Discussion

The aim of this thesis was to examine the significance of MND in healthy children born at term without risk for developmental disorders. To this end, we specifically studied whether MND in infancy was associated with parental psychological well-being and anxiety and whether MND at school age was related to IQ, cognition and behaviour. In the second chapter, we demonstrated that maternal trait anxiety was associated with a less optimal neurological development of the infant. In the following chapters we showed that school aged children with MND, and in particular with fine manipulative disability and coordination problems, are at risk for developing cognitive problems - specifically problems with attention, memory and learning and language - and externalizing behaviour. The associations between MND and cognition and behaviour appeared to be sex specific. In boys, fine manipulative disability was associated with lower IQ scores, whereas this association was absent in girls. On the other hand, in girls, particularly coordination problems and dysfunctional posture and muscle tone were related to cognitive deficits and behavioural problems, whereas this was not the case in boys.

In this discussion I will first give some methodological considerations. Next, the discussion will focus on the possible underlying substrate for neurocognitive and neurobehavioural associations. Specific attention will be paid to the possible role of stress during early life for neurocognitive and neurobehavioural development and the sex differences in these relations. Finally, suggestions for future research will be given as well as concluding remarks.

Methodological considerations

In this thesis, two different projects were used to examine the neurocognitive and neurobehavioural associations. These projects were both originally designed for other purposes, which had consequences for the composition of the study groups.

The Groningen ART cohort

In the Groningen ART cohort all children had been born to parents with a history of subfertility. Furthermore, part of the children were born following assisted reproductive technology. Assisted reproduction, as well as a history of subfertility, have been associated with adverse perinatal outcome, such as preterm birth and low birth weight (1-3), which in turn are associated with developmental disorders (4,5) and minor neurological dysfunction (6,7). Furthermore, subfertility and IVF treatment also may influence well-being in couples (8), therefore causing a potential bias in our study, and implying that the generalisability of the results is limited. However, both infant neurological development and parental well-being and anxiety were not affected by assisted reproduction in the present study group (9,10).

The information on parental psychological well-being and infant neurological condition was collected at different time points; during this time, parents of 10 children decided not to participate in the follow-up at 12 months of age. However, participants and non-participants at 12 months did not differ on background variables, such as parental age and education and infants gestational age, birth weight, sex and neurological optimality at 10 months. Therefore, it can be assumed that this attrition did not influence the results. However, the time difference in data collection of parental psychological well-being and infant neurological classification, may be regarded as a limitation of the study.

Also, it is more difficult to interpret the association between two measures when they are assessed at different time points. Furthermore, it would have been interesting to collect data on parental anxiety and well-being at different time points, including various prenatal assessments, to gain more information regarding the point in time at which maternal anxiety may influence neurological development of the infant. However, the study included a measure of trait anxiety, which is a relatively stable attribute of people to perceive situations as frightening and react with increased anxiety to these situations. It can be assumed that trait anxiety as measured by the STAI remains constant over time, as is reflected by a high test-retest reliability (11).

Information regarding parental well-being and anxiety was collected using self-assessment questionnaires. Self-reports may be vulnerable to social desirability, leading to biased and lower scores on the questionnaires. This may be reflected by the low anxiety scores in this study: only 3% of the mothers that filled in the questionnaire had anxiety scores higher than one standard deviation above the mean of the norm population (12). However, other explanations for the low anxiety scores found in this population can be given. For example, the strict selection of subfertile couples to become eligible for ART may already provide a selection bias for couples that are more (psychologically) capable of undergoing ART treatment. This is supported by a report that shows psychological distress to be a reason for discontinuing IVF treatment (8), thereby creating a selection bias. Also, it has been demonstrated that the negative emotional feelings associated with infertility (13) disappear after successful treatment outcome (14,15) .

Despite these limitations, the study offered valuable information regarding the association between maternal anxiety and infant neurological development. In particular, the fact that both maternal and paternal psychological health was assessed is a strong point of this study. A second strength includes the use of a detailed neurological examination, which not only measures domains of dysfunction, but also neurological optimality - the number of items for which optimal performance is achieved. The range for optimality is more narrow than the range for normality, making this a very sensitive outcome measure. It can be expected that the association between parental well-being and anxiety and infants neurological development is subtle; the use of a detailed neurological examination made it possible to detect this relation.

The Groningen LCPUFA project

The Groningen LCPUFA project was also originally designed for other purposes, thereby influencing the composition of the study group. The Groningen LCPUFA study is a randomized controlled trial on the effects of supplementation of infant formula with long chain polyunsaturated fatty acids (LCPUFA). To this end, the children of parents who opted for formula feeding after birth, were randomized to receive either formula supplemented with LCPUFA or control formula. A third group of infants was breastfed after birth, serving as a control group to the formula groups. This design influenced the study group composition in several ways. First, as a consequence of the allocation to different feeding groups, a smaller percentage of children were breastfed after birth compared to the Dutch population: 37% as opposed to 78% of children were breastfed (16). This may have influenced results, as breastfeeding is associated with social and biopsychosocial factors, as well as with neurological and cognitive development and behaviour. For example, breast feeding in the Netherlands is associated with higher paternal and maternal education, maternal non-smoking and full time profession, and infant gestational age (more than 38 weeks) and parity (firstborn) (16). With respect to neurodevelopmental outcome, breastfeeding has been associated with better neurological (17,18) and cognitive development (19), higher IQ (20,21), better social competence and less attention problems (22). However, this may also be due to other factors associated with breastfeeding, such as maternal IQ or parenting skills (23). The low rate of breastfeeding in this study group may (partially) explain the high prevalence of MND. In this thesis, prevalences of 37% for simple MND and 13% for complex MND are reported, whereas previously the prevalence of simple MND was reported to be 15-20% and the prevalence of complex MND 6-7% (24,25). With respect to the specific domains of dysfunction, we found the following prevalences: 47% dysfunctional reflexes, 27% coordination problems, 25% fine manipulative disability, 18% dysfunctional posture and muscle tone, 4% choreiform dyskinesia, and in 2% of children excessive associated movements. Compared to previously published prevalences, this thesis reported particularly high prevalences of coordination problems and fine manipulative disability (25,26). Formula feeding is associated with a delay in motor coordination (27) and fine manipulative disability (18). Due to the composition of our study group and the relatively low amount of breastfed children, this may offer an explanation for the high prevalence of coordination problems and fine manipulative disability, and consequently of MND. Furthermore, the low prevalences reported by Peters et al. (25), are found in children visiting a mainstream school. The study group in this thesis also included children receiving special education (6% of participating children). The prevalence of simple MND and complex MND is much higher in children visiting special education (40% and

53% respectively) (25). This may be explained by an increase in coordination problems and fine manipulative disability in children receiving special education: the prevalence of dyscoordination is 83% and of fine manipulative disability 86% (25). The difference in composition of the study groups may thus also explain in part the high prevalences found in this thesis. The high prevalence of MND reported in this thesis may also in part be a reflection of a general increase in developmental motor problems (28). A final explanation may be that parents of children who appear clumsy have a stronger motivation to participate in a study on neurodevelopment. This may also be the case for parents with children with ADHD, as the study group also had a relatively high prevalence of children with a diagnosis of ADHD (9%) compared to previous reports (29,30). However, this too may reflect changes and increases in diagnostic practices over the years (31).

The results reported in this thesis may also have been influenced by the selective attrition in the LCPUFA project: children were enrolled at birth and follow-up took place at 3 months, 18 months, and 9 years postpartum. In this thesis, only results of the follow-up at nine years are reported, at which time 72% of the children participated. Characteristics of participating and non-participating children were comparable with respect to gestational age, birth weight, Apgar scores, sex, parity, parental education and profession and maternal smoking, alcohol and drug use during pregnancy. However, there were differences between participating and non-participating children regarding type of feeding after birth and quality of general movements at 3 months of age: children breastfed after birth and children with normal-optimal GMs more often participated at nine years (breastfeeding: 37% vs 24%; GMs: 28% vs 14%). Children fed formula supplemented with LCPUFA participated less at follow-up at nine years of age (participants: 27%; non-participants 41%).

A final limitation of the study may be that the assessors were not blinded to cognitive outcome when carrying out the neurological assessment. This potential bias may have been cancelled out by the independent assessment of the neurological condition by an assessor who remained blinded to cognitive outcome. Therefore, it is unlikely that the results of the cognitive assessments influenced the evaluation of the neurological examination.

In the study group only healthy, term born children were included. This may be regarded as a strength, as the studies have helped to gain important information on neurodevelopment in typical children, without perinatal or neonatal risk for developmental disorders. Furthermore, due to the enrolment of children at birth, information regarding obstetric events were accurately recorded and due to the follow-up of the group, many background and environmental factors during childhood and at nine years of age were available. Also, the neurological examination used, the examination of the child with MND, can be considered a strength, as it is a detailed assessment, which takes age-related performance into account. The examination al-

lows for the assessment of subtle dysfunctions across various domains. This makes the examination very useful for assessing neurological status in typical children, in whom evident pathology is not expected.

The neurological substrate for neurocognitive and neurobehavioural associations

Chapters 3-5 of this thesis report associations between MND on the one hand and lower IQ, impaired cognition and behavioural problems on the other hand. These associations may be attributed to dysfunction of complex cortical-subcortical brain networks, which subserve motor functions as well as cognition and behaviour. The cortico-striato-thalamo-cortical and cerebello-thalamo-cortical pathways are proposed candidates underlying the associations between MND, IQ, cognition and behaviour. The cortico-striato-thalamo-cortical pathway is involved in sequential motor learning, for example in sequential finger movement tasks (32,33), which suggests a role in fine manipulative ability, whereas the cerebello-thalamo-cortical pathway is in particular of importance in the initial phases of motor learning and timing of movements (33,34) and motor coordination (35). Subcortical structures, such as the cerebellum also play an important role in regulation of posture and muscle tone, although the regulation of postural control is complex and requires cooperation of major parts of the central nervous system (36-38). The abovementioned brain regions are not only involved in motor functions, but activation of the cortico-striato-thalamo-cortical and cerebello-thalamo-cortical networks is also required in cognitive functions. The first network is involved in spatial working memory, planning and attentional switching (32) and the latter in planning, language and set-shifting, respectively (34,39). Finally, functioning of these structures is associated with behaviour, as is illustrated by the association between increased volume of the basal ganglia, anterior cingulate cortex and orbitofrontal cortex and more aggressive behaviour in children (40). In addition, smaller hippocampal volume has been related to more withdrawal and anxiety in typically developing children (9). Due to the wide variety in functions subserved by these cortical-subcortical brain networks, it can be assumed that dysfunction of these networks is manifested in impairments across all domains: motor function, cognition and behaviour.

In the studies presented in this thesis, we demonstrated not only associations between MND and IQ, cognition and behaviour, but we also demonstrated that these associations may be sex-specific to some extent. The results showed that in particular girls with coordination problems or dysfunctional posture and muscle tone were at risk for cognitive impairment and behavioural problems; a similar vulnerability was absent in boys. However, boys with fine manipulative disability had lower IQ scores,

whereas girls with fine manipulative disability were not at risk for lower IQ scores. The association between simple MND, with it's weak relation to perinatal adversities, and unspecific behavioural problems in boys point towards a relatively important effect of psycho-social factors in the genesis of behavioural problems in boys.

The manifestation of sex-specific neurocognitive and neurobehavioural associations can be explained by differences in development of the underlying cortico-striato-thalamo-cortical and cerebello-thalamo-cortical pathways. As described in the introduction, the female brain has increased cortical thickness of the right lateral parietal and temporal regions (41) and larger volume of the hippocampus and the caudate nucleus of the basal ganglia compared to the male brain (42,43). Boys, on the other hand, have relatively larger amygdala (42) and cerebellum (44,45). Consequentially to these sex differences in development, it may be that girls are more vulnerable to dysfunction of the cerebellum and amygdala, making girls more susceptible to deficits in the functions subserved by these brain areas, such as coordination problems, dysfunctional posture and muscle tone and the associated impairments in cognitive function and behaviour. In contrast, boys may be more susceptible to dysfunction of the basal ganglia and hippocampus, manifesting in fine manipulative disability and its associated impairments.

Stress during early life and neurological development

MND has been related to stress during early life, such as preterm birth or intrauterine growth restriction (6,46,47). During early life, and in particular during fetal life, the nervous system is subjected to rapid development, making the fetal nervous system vulnerable to environmental influences. In the second chapter of this thesis we demonstrated that maternal trait anxiety – a form of psychological stress - was associated with a less optimal neurological condition in the infant. As paternal trait anxiety was not associated with infant neurological condition, we assumed that the association has a biological nature, which may already come about during fetal life. This is supported by studies on the relation between maternal prenatal stress and neurodevelopment: prenatal stress has been associated with behavioural, cognitive and motor development in childhood. Behaviour associated with prenatal stress includes difficult temperament (48), fearfullness (49), inattention/hyperactivity, emotional problems and conduct problems (50); impaired cognitive development is reflected by lower scores on the mental development index of the Bayley Scales of Infant Development (BSID) (49,51,52) and on productive and receptive language abilities (51). Finally, prenatal stress may influence infant motor development (52).

What might be the mechanism through which stress during early life influences neurodevelopment? First, the possible contributing mechanisms will be de-

scribed: programming of the hypothalamo-pituitary-adrenal (HPA) axis, alterations in the monoaminergic systems and structural changes in response to prenatal stress. Then evidence for a sex-specific nature of these mechanisms will be given.

Stress during early life may influence several aspects of neurological development. Firstly, maternal stress and cortisol levels may induce changes in the fetus through programming of the HPA axis. Maternal stress hormones can influence the fetal HPA axis though several ways. One explanation may be that maternal stress hormones are transported across the placenta to the fetal circulation, causing a rise in fetal stress hormones (53). An alternative is that maternal stress hormones influence the uteroplacental blood flow, due to their constrictive effect on vessel tone (54), resulting in reduced blood flow and supply of nutrients to the fetus. The reduced availability of nutrients and oxygen may directly influence growth and development, or indirectly via activation of the fetal HPA axis. A final explanation may be that the maternal stress increases production of placental corticotropin-releasing hormone (55), which in turn can activate the fetal HPA axis. The effects of maternal stress on the child's stress response may last until adulthood. Children exposed to prenatal stress have an altered stress response, although results are not consistent: one study reported a lower cortisol but higher ACTH response in young adults after a stress test (56), whereas another study reported increased cortisol levels after exposure to a stressor in infants (57). Studies have, however, consistently demonstrated raised diurnal cortisol levels and higher basal cortisol levels in infancy and childhood (58).

The elevated levels of glucocorticoids in infancy may influence brain development: in prenatally stressed rats changes have been demonstrated in receptors of importance for the regulation of the HPA axis and in the monoaminergic system of the brain. It has been demonstrated that the hippocampal density of the mineralocorticoid receptor is increased, whereas the hippocampal density of glucocorticoid receptors is decreased in rats (59); the alterations of receptor density can then influence HPA axis regulation. Animal studies also suggest that stress during early life also induces changes in the monoaminergic systems of the basal ganglia, cerebellum, hippocampus and frontal cortex, which are associated with MND (24,60). These changes are regionally specific, for example reduced levels of norepinephrine and dopamine are found in the locus coeruleus, reduced levels of norepinephrine and serotonin in the cortex, and reduced levels of dopamine in the striatum and nucleus accumbens. In the prefrontal cortex, increased dopamine turnover has been demonstrated in animals exposed to prenatal stress (61). Altered monoaminergic function in widespread brain regions may also manifest itself in altered motor, cognitive or behavioural function. The changes in neurochemistry may result in structural changes in the brain after exposure to prenatal stress due to an influence on neurogenesis. In monkeys, prenatal stress has been associated with reduced hippocampal volume and reduced

neurogenesis in the dentate gyrus (62). Furthermore, in prenatally stressed rats, learning-induced hippocampal neurogenesis is also inhibited (63), whereas the lateral nucleus of the amygdala is enlarged in prenatally stressed rats (64). Neuroimaging studies have offered the opportunity to examine structural changes in humans after exposure to prenatal stress: prenatal anxiety was associated with smaller volumes of the gray matter in the prefrontal cortex, the premotor cortex, the medial temporal lobe, the lateral temporal cortex and the cerebellum in children aged 6-9 year (65).

The manifestation of structural and functional alterations associated with prenatal stress have led to an interest for a possible moderating influence of timing of the stressor. In the second chapter, we hypothesized that the association between maternal trait anxiety and infant neurological condition approximately one year after birth had a biological nature, with a possible prenatal origin. However, we did not have information on maternal anxiety during pregnancy; consequently conclusions of a temporal effect of maternal anxiety on child development could not be drawn. Nevertheless, other studies on prenatal stress and child development have paid specific attention to the timing of the stressor. It is generally agreed upon that the timing of stress during pregnancy influences outcome, although inconsistent results have been published on the nature of the temporal vulnerability. Maternal anxiety during the first trimester has been associated with ADHD-symptoms (66). Most studies however, have focused on maternal anxiety in the second and third trimester. Van den Bergh et al. demonstrated effects of maternal anxiety between 12 and 22 weeks of gestation on behaviour in 8 and 9 year old children and cognition in 14 and 15 year old children; in these children, prenatal stress occurring between 23-31 or 32-40 weeks of gestation was not associated with neurodevelopmental outcome (67,68). This was in part confirmed by Huizink et al, who demonstrated that anxiety during the second trimester was associated with lower mental and motor development in infants; however, they also found that raised maternal cortisol levels in the third trimester of pregnancy was associated with the infant's mental and motor development (52). Other studies too demonstrated associations between maternal anxiety in the third trimester and child behavioural problems and cognition (50,69,70); however, in these studies the associations with outcome at 4 years of age were stronger for anxiety in the third trimester compared to maternal anxiety in the second trimester (50,70). Differences in the effects of timing may be due to the designs of the studies, the type and duration of the stressors, type of developmental outcome, child's age at follow-up and the confounders that were adjusted for.

It is hypothesized that the influence of temporal effects of stressors is associated with the ontogeny of the nervous system. Animal studies have demonstrated that prenatal stress influences hippocampal neurogenesis (62) and induces synaptic loss (71). These processes peak during the second and third trimester of pregnancy, respectively (72), the above mentioned periods during which prenatal stress may

have long term neurobehavioural sequelae. During this time, neurotransmitter systems, such as the catecholaminergic and GABA-ergic systems, also go through rapid development (72) – systems which are influenced by prenatal stress (60).

Sex specific reactions to stress

The associations of prenatal stress with specific neurological processes have also led to a focus on sex specific reactions to prenatal stress, as sex differences exist in typical neurological development of structures associated with the stress response - the monoaminergic systems, hippocampus, basal ganglia and amygdala –(73). Therefore, it is conceivable that sex differences may also occur in response to adverse events. This is supported by a sex-specific reaction to stress of the monoaminergic and GABA-ergic systems in rats. In male rats, prenatal stress has been associated with decreased hypothalamic noradrenaline levels (74) and decreased dopamine and serotonine levels (75), whereas in female rats, prenatal stress is associated with increased dopamine levels (75). Furthermore, neonatal stress in rats influences the typically occurring switch of the GABAa-ergic system from depolarizing to hyperpolarizing state in a sexual dimorphic manner. Typically, GABAa-ergic signaling is depolarizing in early postnatal life, a situation which gradually shifts to hyperpolarizing. The timing of this switch is sex specific: female rats switch to hyperpolarizing mode earlier than male rats (76). In male rats stress causes an acceleration of the switch of the GABAa-ergic system to hyperpolarization, whereas in female rats, it induces a reappearance of the depolarizing state of the GABAa-ergic system (76). Sex differences also occur with respect to programming of the HPA axis. In prenatally stressed females, increased response of the HPA-axis has been demonstrated, whereas male offspring had slightly reduced levels of ACTH (77).

The sex-specific alterations in programming of the HPA axis of the monoaminergic and GABA-ergic systems may result in a sexually dimorphic vulnerability for developmental disorders. Althought we did not find an interaction between sex and maternal anxiety for infant neurological outcome, it may be that the sex differences in the sequelae of adverse events, such as stress during early life, manifest itself at later ages as the child is challenged to perform more complex tasks.. Previously, sex differences in response to adverse events have been demonstrated. For example, an increased risk for schizophrenia has been found for men in association with prenatal stress occurring in the second trimester of pregnancy (78,79), and an increased risk for women in association with prenatal stress during the first trimester (80). Most evidence for sex specific vulnerability for developmental disorders, however, comes from animal studies. In rats, prenatal stress has been associated with increased anxiety-like behaviour (75,81) only in female offspring. Conversely, in male rats, exposure to prenatal stress was associated with impaired learning ability (82,83).

The above mentioned sex differences in the HPA-axis and brain neurochemistry in response to prenatal stress may underlie the sex differences in cognitive and behavioural impairments associated with prenatal stress. However, the exact manner in which this comes about remains unclear, although a role for the gonadal hormones has been proposed, as sex steroids can influence programming of the HPA axis and development of the nervous system. Estrogens enhance the response of the HPA axis, whereas testosterone inhibits the HPA axis response (84), which is in line with the findings of the sex-specific HPA axis response described above. The focus of research on the role of gonadal hormones as modulators of a sex specific stress response has been on testosterone due to the finding that fetal cortisol levels are positively related to fetal testosterone levels (85). Development of the nervous system is also influenced by fetal testosterone, as testosterone is associated with regional brain volumes and with alterations in the catecholaminergic systems. For example, higher levels of testosterone are related to larger volume of the gray matter of the right temporoparietal junction/posterior superior temporal sulcus and smaller volume of the gray matter of the planum temporale/parietal operculum and posterior lateral orbitofrontal cortex (86). Development of catecholamines is modulated by testosterone via its conversion to estradiol (87). The influence of fetal testosterone on brain development is further reflected by associations between fetal testosterone and neurodevelopmental outcome. It has been demonstrated that fetal testosterone can predict sexually differentiated behaviour (88) and cognition, for example spatial ability (89) and language development (90). In addition, higher levels of fetal testosterone are associated with autistic traits (91) and play a role in the etiology of ADHD/ODD and PDD-NOS (92).

If prenatal stress, reflected by increased levels of fetal cortisol, is associated with increased levels of testosterone, then it is plausible that alterations occur in development of the brain areas and catecholamines associated with testosterone. This effect may be augmented as prenatal stress is related to decreased activity of steroid aromatase, thereby influencing conversion from testosterone to estradiol (93) and influencing catecholamine levels (87). Since the typically developing brain responds to testosterone levels in a sexually dimorphic manner, it is also plausible that the sex differences occur as a manifestation of higher testosterone levels in response to prenatal stress. The developing male brain may be more vulnerable to higher levels of testosterone, due to their already higher levels in typical development (88) and due to higher levels of androgen receptors in the male brain (94). This hypothesis is supported by a study that demonstrated that higher levels of prenatal testosterone in girls was associated with higher scores on a mental rotation task, whereas in boys, higher levels of prenatal testosterone were related to lower scores on this task; high levels of prenatal testosterone in girls, remained lower than the lowest levels of testosterone on boys (95). Further research regarding the role of gonadal hormones and prenatal stress is warranted.

Epigenetics in development and developmental disorders

In the last decade increasing attention is paid to the role of epigenetics, meaning a complex interaction between genes and environment, in the etiology of developmental disorders, for example ADHD (96). Environmental factors can alter gene function through processes such as DNA methylation or histone modification. The modification of gene expression can explain how exposure to adverse events can have long-term sequelae. Furthermore, epigenetic modifications as a result of environmental factors may explain why exposure to the same adverse events result in different outcome in offspring.

The above described association between early life stress and later neurodevelopmental outcome may also come about due to epigenetic modifications; epigenetic mechanisms have previously been suggested to play a role in the origin of simple MND (24). This can offer an explanation for the association between simple MND and unspecific behavioural problems in boys demonstrated in this thesis: psychosocial factors can interact with genes resulting in a long-term behavioural problems. It has been demonstrated that exposure to prenatal maternal anxiety is associated with increased methylation of the human glucocorticoid receptor gene, which in turn was associated with increased reactivity of the HPA axis to stress (97). Further evidence for a epigenetic origin of the neurodevelopmental sequelae of prenatal stress comes from a study demonstrating that increased cortisol exposure affects gene expression in human fetal brain cells (98). Genes contributing to cell growth, dendritic morphogenesis, receptor functions and intracellular signaling were overexpressed, whereas other genes related to cell cycle and proliferation, protein catabolism, apoptosis, cellular organization, immune response, and intracellular signaling were underexpressed (98). Due to these changes in gene expression, prenatal stress can have long-lasting effects, and this may explain later susceptibility for developmental disorders. Epigenetic alterations in response to perinatal adversities can also offer an explanation for sex differences in developmental disorders. There is evidence that sex specific epigenetic processes are regulated by the gonadal hormones estrogen and estradiol, which is converted from testosterone (99). Sex specific epigenetic regulation has been demonstrated in mice with increased placental gene expression in male placentas only and not in female placentas (100). It can be hypothesized that prenatal stress alters typical sex differences in epigenetic regulation, resulting in a sex specific vulnerability for prenatal adversities.

Although the focus of this discussion has been on prenatal stress, other factors associated with developmental disorders later in life, may also induce epigenetic modifications, such as maternal smoking (101) or alcohol use during pregnancy (102) and maternal diet (103). In this thesis, the study groups consisted of children born to subfertile couples (the Groningen ART cohort) or children participating in a trial on

the effects of long chain polyunsaturated fatty acids (the Groningen LCPUFA project). As was mentioned earlier on in this discussion, these factors of the study group composition also influenced developmental outcome. Is it possible that epigenetic alterations play a role in these associations as well? In the Groningen ART cohort, it has been demonstrated that increased time to pregnancy, an indication for subfertility, is related to an increased risk for MND at two years of age (104), implying that the factors associated with subfertility, such as the genetic constitution or altered hormonal milieu, play a role in neurodevelopmental problems. As we mentioned above, estrogen and testosterone, via its conversion to estradiol, influence gene expression; is it then possible that an altered hormonal milieu in subfertile parents influence child neurodevelopmental outcome by inducing epigenetic alterations?

Similarly, type of feeding after birth has been associated with neurodevelopmental outcome: children breastfed after birth less often had MND or fine manipulative disability compared to children formula fed after birth (17,18). It is assumed that the long chain polyunsaturated fatty acids (LCPUFA) present in breast milk, but previously not in formula, play a role in the beneficial effect of breast milk on neurological development. Results of the Groningen LCPUFA project demonstrated a beneficial effect of breast feeding compared to formula on neurological development; children fed formula supplemented with LCPUFA had a comparable neurological development to children fed control formula at nine years of age (18). With respect to cognition the effects of LCPUFA were not consistent. In healthy children, LCPUFA supplementation was associated with lower performance on executive function. Conversely, a beneficial effect of LCPUFA was demonstrated on verbal IQ and learning and memory; the positive effect however was dependent of maternal smoking during pregnancy. Behaviour was not influenced by LCPUFA supplementation at nine years (21). A review on the effect of LCPUFA supplementation concluded that LCPUFA appeared to have a positive effect on neurodevelopmental outcome until the age of 4 months (105). This effect may come about as LCPUFA are essential components of the nervous system: in infant baboons, high levels of LCPUFA are found particularly in the cerebral cortex and basal ganglia (106). Subsequently, LCPUFA are likely to influence functions subserved by brain circuitries involving these brain regions, such as motor learning, cognition and behaviour (32,33). The effect of LCPUFA may also come about due to epigenetic alternations, as LCPUFA have been demonstrated to influence neuronal gene transcription in rats (107).

In conclusion, many environmental factors can influence neurodevelopmental outcome in the child. Factors mentioned include stress and nutrition during early life, maternal alcohol use and smoking during pregnancy, subfertility and assisted reproduction. It is possible that epigenetic alterations are the underlying substrate to the long lasting effects of these factors on neurodevelopmental outcome. However, the precise mechanisms remain unclear and deserve further attention in research.

Suggestions for future research

The manifestation of minor neurological dysfunction is age-dependent, meaning that the prevalence varies across different age groups. In this thesis, results are reported of infants and children aged nine years. For future research it may be interesting to examine children after puberty, for example at 14 years of age. At nine years of age, the prevalence of MND is relatively high, which is thought to be the result of increasing demands on and complexity of brain functions. Although higher order cognitive functions are still developing and increasing in complexity in adolescence, the prevalence of MND decreases after puberty (108). It was proposed that the hormonal changes occurring during puberty are responsible for this decrease through their influence on neurological development (108,109). For example testosterone levels during puberty have been associated with volumes of the hippocampus and amygdala (110). The gonadal hormones may especially influence the brain circuitries thought to underlie minor neurological dysfunction, i.e. the cortico-striato-thalamo-cortical and cerebello-thalamo-cortical circuitries, as these brain circuitries are still developing in adolescence. This is supported by previous work of Soorani-Lunsing, demonstrating that puberty influenced the prevalence of neurological problems (108). Neurobehavioural associations were also influenced by the onset of puberty (111). Soorani-Lunsing et al. studied a population in part consisting of at risk children. Associations between specific learning and behavioural problems and MND have not been studied in healthy, adolescents born at term and therefore follow-up on neurocognitive and neurobehavioural associations in children after the onset of puberty is warranted.

The interaction between MND and sex also deserves further attention. As the study was not set up to detect sex differences in neurocognitive and neurobehavioural associations, it is likely that the studies did not have enough power to detect sex differences in these associations. For example only 31 boys were classified with complex MND and only 14 girls were classified with complex MND. As a result, interactions between MND and sex may have failed to reach significance due to a lack of power. Therefore, it would be interesting to replicate the results in a larger group of healthy term born children. It would also be interesting to complement the examination of minor neurological dysfunction with neuroimaging. This may provide more information on the involved brain circuitries and on sexual dimorphism in the established neurocognitive and neurobehavioural associations.

The hypothesis that stress during early life may result in the manifestation of MND and its associated impairments also deserves further attention in future research. Long term follow-up of children with the availability of repeated prenatal and

postnatal measurements of maternal stress may shed further light on timing effects of maternal stress, subsequent child development and indications for interactions with sex in neurodevelopment.

Concluding remarks

Minor neurological dysfunction is often found in children presenting with motor problems (25). Therefore, the findings reported in this thesis may have implications for clinical practice: in children presenting with motor problems, it is advisable to perform the examination of the child with MND, as the presence of MND indicates the child's vulnerability for co-occurring cognitive and behavioural problems. Thus, if MND is present, screening for behavioural and cognitive problems is recommended, as the results of this thesis indicate that children with MND are at increased risk for developing cognitive and behavioural problems. The screening for cognitive and behavioural problems in children with MND may aid in early identification of problems. In doing so, consideration should be given to sex differences in brain development: the results indicated that in particular girls with coordination problems or dysfunctional posture and muscle tone are at increased risk for associated cognitive and behavioural problems. On the other hand, in particular boys with fine manipulative disability are at risk for associated problems. However, further research is needed to clarify sexual dimorphism in the significance of minor neurological dysfunction.

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Chapter 7

Summary

Over the last decade increasing attention has been paid to developmental disorders and their underlying neural substrate. This is in part the result of advances in imaging studies. Consequently, this has led to increasing attention for minor neurological dysfunction (MND), which is the occurrence of neurological dysfunction in absence of apparent pathology, such as cerebral palsy. Manifestations of MND include subtle coordination problems, fine manipulative disability and dysfunctional posture and muscle tone regulation.

A prominent part of typical neural development, consisting of neuronal proliferation, migration and organization of the nervous system, takes place during fetal life. But also after birth substantial developmental changes occur continuing until adulthood, with regional differences. For example the hippocampus and amygdala reach peak sizes between 9 and 11 years of age, the prefrontal cortex at approximately 11.5 years of age and the cerebellum between 12 and 15 years of age. This is in line with functional brain development. Brain areas that mature first are associated with basic sensory and motor functions. Areas that show prolonged developmental changes are associated with complex cognitive and motor functions, such as language, working memory and motor coordination. Interestingly, it is getting increasingly clear that sex differences exist in typical brain development. In general, girls reach peak sizes of regional brain volumes before boys. With respect to brain volume, boys generally have a larger overall brain volume, a larger amygdala and cerebellum, whereas girls have relatively larger basal ganglia and hippocampus. These sex differences in typical development may also have implications for developmental disorders. This notion is supported by the well known sex differences in the prevalence of developmental disorders.

In developmental disorders, motor problems frequently co-occur with problems in other neural domains, such as cognition and behaviour. This has led to increased attention for MND. MND can be described in severity and type of dysfunction. The type of dysfunction may be described in eight different domains of dysfunction: fine manipulative ability, coordination, choreiform dyskinesia, posture and muscle tone, reflexes, associated movements, sensory deficits and cranial nerve function. The severity of MND is described in simple MND or complex MND. At school age the difference is based on the number of dysfunctional domains. Simple MND, implying one or two domains of dysfunction, can be regarded as a non-optimal form of development, whereas complex MND, in which more than two domains of dysfunction are present, is regarded as the clinically relevant form of MND. This thesis aims to contribute to knowledge on the clinical significance of MND, which is of importance as an understanding of co-occurring problems may also provide opportunities for intervention.

The following questions are addressed in this thesis:

1. Are parental psychological distress or anxiety associated with MND in healthy noenates?
2. Is the presence of MND in healthy term born 9-year-olds associated with IQ, cognition and behaviour? Are specific types of MND associated with cognition and behaviour? Do sex differences occur in the associations between MND and IQ, cognition and behaviour?

The questions were answered on the basis of two groups of children. For the first question, 206 healthy neonates from the Groningen ART cohort, a study designed to examine relationships between assisted reproduction and neurodevelopmental outcome, were neurologically assessed at the age of 10 months. Information on parental anxiety and well- being was collected at the infant's age of 12 months.

The Groningen LCPUFA project enabled us to answer the second question. This project was designed to examine effects of supplementation of formula with long chain polyunsaturated fatty acids (LCPUFA) during the first two months after birth.At nine years of age, 341 children were neurologically examined; the children also performed an IQ test and cognitive tests in the domains of attention, learning and memory and language. Parents and teachers were asked to evaluate the child's behaviour at nine years of age by means of a questionnaire.

1. Are parental anxiety and well-being associated with minor neurological dysfunction in infants without perinatal risk?

The results of chapter 2 indicated that infants born to mothers with high trait anxiety are at increased vulnerability to develop a non-optimal nervous system. Paternal anxiety and well- being are not associated with infant's neurodevelopmental outcome. Therefore, the results imply a prenatal, biological effect of maternal anxiety on infant neurological condition. This association may come about through programming of the infant's hypothalamo-pituitary- adrenal axis and through early programming of the monoaminergic systems, which are involved in sensorimotor function, movement preparation and execution and coordination.

2. Are cognition and behaviour associated with minor neurological dysfunction in nine year old children born at term? Are the associations sex-specific?

The second part of this thesis demonstrated that MND is associated with lower IQ scores, cognitive impairments and behavioural problems. The results of chapter 3 and 4 demonstrated that in particular the type of MND is important: coordination problems and fine manipulative disability were associated with lower IQ

scores and cognitive impairments in the domains of attention, memory and learning and language. It is hypothesized that dysfunction of complex brain circuitries, i.e. the cortico-striato-thalamo-cortical and cerebello-thalamo-cortical networks, underlie the association between the types of MND and cognitive impairments, as these networks are required in motor function as well as cognition. The results also suggested a sex-specific nature of these neurocognitive associations: in particular girls with coordination problems and dysfunctional posture and muscle tone were at risk for cognitive impairments. This vulnerability differed from the vulnerability in boys, as in particular boys with fine manipulative disability were at risk for lower IQ scores. The manifestation of sex-specific neurocognitive associations may be the result of differences in development of the underlying cortico-striato-thalamo-cortical and cerebello-thalamo-cortical pathways.

In chapter 5 the results of associations between MND and behaviour are reported. This chapter supported evidence for sex-specific associations. Girls with complex MND, and specifically with dysfunctional posture and muscle tone, were at increased risk for developing externalizing behavioural problems. This corroborates the hypothesis of the involvement of dysfunction of complex brain circuitries, i.e. the cortico-striato-thalamo-cortical and cerebello-thalamo-cortical networks, as these brain circuitries are activated in motor tasks as well as behaviour. The specific vulnerability of girls with dysfunctional posture and muscle tone regulation for externalizing behavioural problems differs from the unspecific vulnerability in boys: in boys simple MND, i.e., the presence of a typical, but non-optimal neurological condition, was associated with an increase of unspecified behavioural problems. This implies that in boys psychosocial factors may play a larger role in the origin behavioural problems than in girls.

In conclusion, the results suggest that children with fine manipulative disability and coordination problems are at increased vulnerability for cognitive impairments, and that in particular girls with dysfunctional posture and muscle tone regulation are vulnerable for behavioural problems. Screening for behavioural and cognitive problems in children with MND is therefore advisable as this may aid in early identification of problems. As the results also suggest a sex-specific vulnerability for co-occurring cognitive and behavioural problems, sex of the child should be taken into account. Future research should focus on the implications of MND in children after puberty, as the hormonal changes associated with puberty are know to affect the influence organization of the nervous system, including the manifestation of MND. Thus, the puberty related hormonal changes may also influence neurocognitive and neurobehavioural associations and their sex-specific expression.

Chapter 8

Samenvatting

De laatste jaren is er steeds meer aandacht voor ontwikkelingsstoornissen, zoals ADHD, en hun onderliggende neurale substraat. Dit komt mede door de opkomst van beeldvormende technieken. Het heeft ook geleid tot meer aandacht voor lichte neurologische disfuncties. Lichte neurologische disfunctie (MND) kan gedefinieerd worden als het voorkomen van neurologische disfunctie in afwezigheid van evidente neurologische pathologie, zoals cerebrale parese. MND kan zich uiten in subtiele coördinatie problemen, problemen met fijne handvaardigheid of in de regulatie van houding en spiertonus.

De ontwikkeling van het zenuwstelsel bestaat uit groei, migratie en organisatie van zenuwcellen. Dit proces vindt voor een groot gedeelte plaats voor de geboorte. Maar ook na de geboorte vinden er nog langdurig belangrijke ontwikkelingen plaats in het zenuwstelsel. Dit gaat door tot in de adolescentie. Dit traject van ontwikkeling verschilt voor diverse hersengebieden. Zo bereiken de hippocampus en amygdala hun maximale grootte op de leeftijd van 9 à 11 jaar, terwijl de prefrontale cortex zijn maximale grootte pas bereikt rond 11,5 jaar. In het algemeen geldt dat de gebieden die nodig zijn voor complexe functies later uitontwikkeld zijn dan de hersengebieden die betrokken zijn bij meer basale functies. Er bestaan tevens geslachtsverschillen in de ontwikkeling van het zenuwstelsel, waarbij deze ontwikkeling in het algemeen sneller verloopt bij meisjes dan bij jongens. Jongens daarentegen hebben grotere hersenen en een grotere amygdala en cerebellum dan meisjes. Meisjes hebben echter weer grotere basale kernen en een grotere hippocampus. Deze geslachtsverschillen in hersen ontwikkeling kunnen implicaties hebben voor ontwikkelingsstoornissen. Deze gedachte wordt ondersteund door de welbekende geslachtsverschillen in het voorkomen van ontwikkelingsstoornissen.

Bij ontwikkelingsstoornissen is vaak sprake van problemen op meerdere vlakken: motorische problemen gaan vaak gepaard met problemen in andere domeinen, zoals cognitie en gedrag. Dit heeft geleid tot meer aandacht voor MND. MND kan beschreven worden in termen van het type MND en de ernst van MND. Het type MND kan in acht verschillende domeinen beschreven worden. Voorbeelden daarvan zijn coördinatie stoornissen, problemen met fijne handvaardigheid, problemen met de regulatie van houding en spiertonus, disfunctie van hersenzenuwen of sensibiliteitsstoornissen. Met betrekking tot de ernst van MND wordt onderscheid gemaakt tussen enkelvoudige MND en complexe MND. Op de schoolleeftijd vindt dit onderscheid plaats op basis van het aantal disfunctionele domeinen. Enkelvoudige MND wordt gedefinieerd als de aanwezigheid van één of twee disfunctionele domeinen en wordt gezien als een vorm van normale, maar niet-optimale ontwikkeling. In aanwezigheid van meer dan twee disfunctionele domeinen is er sprake van complexe MND; dit wordt gezien als de klinisch relevante vorm van MND. Dit proefschrift

heeft als doel bij te dragen aan de kennis over en de klinische betekenis van MND. Dit is van belang, omdat kennis over de MND en geassocieerde problemen kan bijdragen aan het bepalen van passende interventie.

De volgende vragen worden besproken in dit poefschrift:

1. Zijn stress en welzijn van de ouders gerelateerd aan MND in gezonde zuigelingen?
2. Is de aanwezigheid van MND gerelateerd aan IQ, cognitie en gedrag bij 9-jarigen zonder perinatale risicofactoren? Zijn specifieke types van MND geassocieerd met cognitie en gedrag? Bestaan er geslachtsverschillen in de associaties tussen MND, IQ, cognitie en gedrag?

Deze vragen werden beantwoord op basis van twee studiegroepen. Voor de eerste vraag werden 206 gezonde zuigelingen van het Groningen ART cohort - een studie over de ontwikkeling van kinderen na hulp bij voortplanting - neurologisch onderzocht. Informatie over stress en welzijn van de ouders werd verkregen op de leeftijd van 12 maanden van het kind.Het Groningen LCPUFA project stelde ons in staat om de tweede vraag te beantwoorden. Dit project onderzocht de effecten van suppletie van flesvoeding met lange keten meervoudig onverzadigde vetzuren gedurende de eerste twee maanden na de geboorte. Op de leeftijd van 9 jaar, werden 341 kinderen uitgebreid onderzocht: zij ondergingen naast een neurologisch onderzoek ook een IQ test en cognitieve testen in de domeinen aandacht, leren en geheugen en taal. Ouders en leerkrachten vulden vragenlijsten in ter evaluatie van het gedrag van het kind.

1. Zijn stress en welzijn van de ouders geassocieerd met licht neurologische disfunctie in zuigelingen zonder perinatale risicofactoren?

De resultaten van hoofdstuk 2 toonden aan dat stress bij de moeder, maar niet haar welzijn, was gerelateerd aan een minder optimale neurologische ontwikkeling van het kind. Stress en welzijn van de vader hadden geen invloed op de neurologische ontwikkeling van het kind. De resultaten van dit onderzoek laten zien dat kinderen van moeders die snel stress ervaren, een verhoogde kans hebben om een niet-optimaal zenuwstelsel te ontwikkelen. Dit duidt op een biologisch effect van stress bij de moeder op de neurologische ontwikkeling van het kind, een effect dat mogelijk al voor de geboorte plaatsvindt, aangezien stress bij de vader niet gerelateerd was aan de ontwikkeling van het kind. Mogelijk ontstaat de associatie tussen stress bij de moeder en een minder optimale neurologische ontwikkeling door middel van een veranderde programmering van de hypothalamo-hypofyse-bijnieras en van de monoaminerge systemen in de hersenen.

2. Is MND gerelateerd aan IQ, cognitie en gedrag bij gezonde 9-jarigen zonder perinatale risicofactoren? Bestaan er aanwijzingen voor geslachtsverschillen in deze associaties?

In het tweede gedeelte van dit proefschrift werd aangetoond dat MND inderdaad geassocieerd is met lager IQ, cognitieve beperkingen en gedragsproblemen. Hoofdstukken 3 en 4 van dit proefschrift toonden aan dat met name het type MND van belang is in de associaties met cognitie: in het bijzonder coördinatie problemen en problemen met fijne handvaardigheid gingen samen met lager IQ en met lagere scores op de cognitieve testen, in de domeinen van aandacht, taal en geheugen en leren. Er wordt gesuggereerd dat disfunctie van complexe hersengebieden , zoals de cortico-striato-thalamo-corticale en cerebello-thalamo-corticale netwerken, aan deze associaties ten grondslag ligt aangezien deze netwerken een rol spelen in zowel motoriek als in cognitie. De resultaten gaven ook aanwijzingen voor geslachtsspecifieke associaties tussen MND en IQ en cognitie. Bij meisjes gaan met name coördinatie problemen en problemen in de regulatie van houding en spiertonus gepaard met cognitieve problemen. Bij jongens daarentegen zijn met name problemen met fijne handvaardigheid gerelateerd aan lagere IQ scores. Deze geslachtsspecifieke associaties zijn mogelijk het gevolg van de geslachtsverschillen in de ontwikkeling van de cortico-striato- thalamo-corticale en cerebello-thalamo-corticale netwerken.

In hoofdstuk 5 werden de resultaten van de relatie tussen licht neurologische disfunctie en gedrag gerapporteerd. Ook dit hoofdstuk geeft aanwijzingen voor geslachtsspecifieke associaties. Meisjes met complexe MND en in het bijzonder met disfunctie van de regulatie van houding en spiertonus hadden een verhoogd risico voor het ontstaan van externaliserende gedragsproblemen. Hiermee wordt tevens de hypothese ondersteund dat complexe netwerken van de hersenen onderliggend zijn aan licht neurologische disfuncties en gerelateerde problemen in gedrag. De kwetsbaarheid van jongens voor gedragsproblemen verschilt van de specifieke kwetsbaarheid in meisjes: in jongens is slechts enkelvoudige MND, dat wordt gezien als een normale, maar niet optimale ontwikkeling van het brein, gerelateerd aan aspecifieke gedragsproblemen. Dit duidt er op dat psychosociale factoren mogelijk een grotere rol spelen in de oorspong van gedragsproblemen bij jongens dan bij meisjes.

Concluderend tonen de resultaten van dit proefschrift aan dat met name kinderen met coördinatie problemen of problemen met fijne handvaardigheid kwetsbaar zijn om ook cognitieve problemen te ontwikkelen. Meisjes met complexe MND zijn tevens kwetsbaar voor het ontwikkelen van gedragsproblemen. Screening van cognitieve en gedragsproblemen in kinderen met MND is daarom te adviseren, daar dit bij kan dragen aan vroege detectie van problemen. Omdat de resultaten ook duiden op geslachtsspecifieke associaties tussen MND en geassocieerde problemen, dient het

geslacht van het kind in overweging genomen te worden bij deze screening. Toekomstig onderzoek zou zich moeten richten op lichte neurologische disfuncties na de puberteit, aangezien de hormonen die gepaard gaan met de puberteit de ontwikkeling van het zenuwstelsel en de manifestatie van MND beïnvloeden. Het is mogelijk dat de associaties tussen MND, cognitie en gedrag en hun geslachtsspecifieke expressie daardoor ook veranderen.

Chapter 9

Dankwoord

Een proefschrift schrijf je niet alleen. De afgelopen jaren heb ik van vele kanten hulp gehad. Soms met directe betrekking tot mijn onderzoek, andere keren in de vorm van steun en een luisterend oor.

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Niet direct bij mijn onderzoek betrokken, maar wel van onschatbare waarde zijn mijn collega's van de ontwikkelingsneurologie. De eerste jaren heb ik vooral veel tips en trucs van de ervarenere onderzoekers gekregen; dank je wel Karin, Kirsten, Marjolein, Cornill en Hylco. Langzaamaan maakten jullie plaats voor nieuwe collega's met wie ik eindeloos veel besproken heb. Niet alleen vragen als "hoe kan ik deze figuur het beste maken", "loopt deze zin", of "zou jij dit manuscript door willen lezen" en de daarbij behorende adviezen, maar ook de gezelligheid bij een kopje koffie met wat lekkers, de wandelingen naar de Albert Hein en het borrelen na het werk maakten dat ik elke dag met plezier naar de afdeling kwam. Dank je wel Lieke van Balen,

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Chapter 10

Curriculum Vitae and list of publications

Hedwig Kikkert werd op 17 februari 1985 geboren te Groningen. Na haar jeugd in het buitenland doorgebracht te hebben, volgde zij haar middelbare schoolopleiding aan het gymnasium Haganum in Den Haag. In 2003 keerde zij naar Groningen terug om te starten met de studie geneeskunde aan de Rijksuniversiteit Groningen. Na het behalen van haar bachelor, legde zij zich toe op haar topsportcarrière in het wedstrijdzwemmen, wat resulteerde in deelname aan de Europese Kampioenschappen in 2008. Tevens rondde zij in 2008 een master Psychologie (richting Hersenen & Gedrag) af, waarvan het afstudeeronderzoek op de afdeling ontwikkelingsneurologie plaatsvond. Het onderzoek bij de ontwikkelingsneurologie werd gecontinueerd in de vorm van een MD/PhD-traject, waarbij de coschappen gecombineerd werden met het promotieonderzoek. Een deel van deze co-schappen deed Hedwig op Curaçao, en bij terugkomst deed zij haar semi-artsstage op de afdelingen Obstetrie en Neonatologie in het UMCG. In 2012 behaalde ze cum laude haar artsexamen. Na een mooie reis naar het buitenland gemaakt te hebben, wil zij zich nu richten op een vervolgopleiding in de richting van de obstetrie en gynaecologie.

List of publications

Kikkert HK, de Jong C, Hadders-Algra M. Maternal anxiety is related to infant neurological condition, paternal anxiety is not. *Early Human Development* 2010;86(3):171-177

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